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ORIGINAL PAPER

JMS Journal of Medical Science

Cardiac dimensions, intraventricular septum thickness in relation to the estimated glucose disposal rate individuals with long-standing type 1 diabetes: a cross-sectional analysis of the PARADISE T1DM study

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ABSTRACT

Aim. Insulin can stimulate the growth of various cells, including cardiomyocytes, through the insulin-like growth factor (IGF) signaling pathway. Insulin resistance (IR), characterized by elevated circulating insu-

lin levels, complicates long-standing type 1 diabetes mellitus (T1DM). This study investigates whether IR in T1DM is associated with cardiac remodeling.

Material and methods. IR was assessed using the estimated glucose disposal rate (eGDR) in 84 adults with T1DM of at least 5 years. Participants with an eGDR at or below the median value were considered more insulin resistant. Transthoracic echocardiography was used to measure left ventricular end-diastolic diameter (LVEDD), intraventricular septal thickness (IVST), right ventricular end-diastolic diameter (RVEDD), and left atrial diameter (LAD). Participants were divided into two groups based on the median eGDR value. Comparisons between groups were made using the Mann-Whitney test.

Results. The median age of the participants was 40.5 years (range: 34.0–51.0), with a T1DM duration of 21.0 years (range: 15.5–27.0) and eGDR of 7.4 (range: 5.1–9.5). Of the participants, 52 (61.9%) were men. Individuals with lower eGDR had larger cardiac diameters (all in cm): LVEDD (4.5 [4.3–4.8] vs 4.75 [4.5–5]; p = 0.01), LVEDD(2.7 [2.5–2.8] vs 2.8 [2.6–2.9]; p = 0.02), end-systolic LAD (3.5 [3.2–3.7] vs 3.8 [3.5–3.9]; p < 0.01), and thicker IVS (0.9 [0.8–1] vs 1.1 [1–1.18]; p < 0.01).

Conclusions. Individuals with T1DM and lower eGDR values exhibited more pronounced cardiac remodeling, with greater LVEDD, RVEDD, and LAD dilation and increased IVST. These findings suggest that people with T1DM at risk of IR have more commonly adverse cardiac structural changes, though causality remains uncertain.

Introduction

Approximately 10% of people with diabetes have type 1 diabetes mellitus (T1DM), which requires lifelong treatment with exogenous insulin injections [1]. Unfortunately, some individuals with T1DM develop insulin resistance (IR) over time, similar to people with type 2 diabetes mellitus (T2DM) [2].

The pathophysiology of IR in T1DM is multifactorial, with contributing factors including obesity, physical inactivity, exogenous insulin treatment, and smoking [2]. The gold standard for assessing IR in T1DM is the hyperinsulinemic-euglycemic clamp [3]. However, the estimated glucose disposal rate (eGDR) is a less time-consuming indirect marker of insulin sensitivity – highly negatively correlated with IR diagnosed with the hyperinsulinemic-euglycemic clamp [4]. IR, as indicated by lower values of eGDR, is associated with the development of macrovascular complications and increased all-cause mortality in T1DM [5,6].

Cardiovascular disease (CVD) is the leading cause of mortality among individuals with T1DM and T2DM [7]. These people often develop left ventricular hypertrophy, diastolic and, over time, systolic heart failure (HF), atrial fibrillation, and ischemic heart disease [8]. One of the proposed pathomechanisms of diabetic cardiomyopathy in T2DM is IR [9]. Liu et al. demonstrated in a prospective cohort study that lower eGDR is associated with future heart failure events in individuals with T2DM [10].

Less is known about echocardiographic changes associated with IR in T1DM. People with T1DM have significantly higher interventricular septal thickness compared to healthy controls [11]. Body mass index (BMI) has been positively associated with left atrial volume and left ventricular mass in adults with T1DM [12]. However, no data on the relation of cardiac dimensions and wall thickness to IR in T1DM individuals are available.

This study compared cardiac dimensions and wall thickness in T1DM individuals with lower and higher eGDR.

Material and methods

Recruitment

This is a post-hoc analysis of a cross-sectional study using data from the Poznań Atherosclerosis in Adult Patients with long-term Type 1 Diabetes Mellitus Study (PARADISE T1DM Study). The Bioethical Committee at the Poznan University of Medical Sciences, Poznan, Poland, reviewed and approved the study protocol (67/19). Written informed consent was obtained from all individuals before inclusion in the study. Each participant was assigned a unique code to ensure anonymity and confidentiality of sensitive and clinical data.

The research was conducted in accordance with the Declaration of Helsinki [13].

Participants were enrolled between February 2019 and March 2020. Inclusion criteria were: age between 18–65 years, T1DM confirmed by positive antibodies with at least a 5-year duration. Exclusion criteria included symptomatic heart failure, left ventricular ejection fraction (EF) below 50%, and moderate to severe valvular diseases. Professional athletes were also excluded. More details on the study protocol, clinical examinations, and measurements can be found in our previous reports [14–16].

For this analysis, we selected data from baseline anthropometric and clinical evaluations, including standard biochemical workups such as lipid profile, thyroid-stimulating hormone, creatinine, transaminases, C-reactive protein, Albumin to Creatinine Ratio (ACR), and HbA1c. Low-density lipoprotein cholesterol (LDL-C) concentration was estimated using the Friedewald formula [15].

IR Assessment by eGDR

The eGDR highly correlates with the results of the euglycemic-hyperinsulinemic clamp, the gold standard for IR assessment in T1DM. The eGDR was derived from the following formula:

$$\text{eGDR [mg/kg/min]} = 24.31 - (12.22 \times \text{WHR}) - (3.29 \times \text{arterial hypertension}) - (0.57 \times \text{HbA1c})$$

where WHR is the waist-to-hip ratio, arterial hypertension is coded as 1 if present and 0 if not, and HbA1c is glycated hemoglobin [%] [4]. Individuals with an eGDR at or below the median value were considered to be less insulin-sensitive.

Transthoracic Echocardiography (TTE)

Resting TTE was performed by a cardiologist using a 3.5-MHz transducer (3Sc-RS phased array ultrasound probe) on a Vivid S6 echocardiography machine from General Electric Healthcare Technologies. Left ventricular end-diastolic diameter (LVEDD), right ventricular end-diastolic diameter (RVEDD), left atrium diameter (LAD) at the end of systole, and intraventricular septum thickness (IVST) at the end of diastole were assessed in the left parasternal long-axis view according to the Guidelines of the Working Group on Echocardiography of the Polish Cardiac Society and the Consensus Document of the European Association of Cardiovascular Imaging [17,18]. Left ventricular EF was measured from the two- and four-chamber apical views using the biplane Simpson's method.

Data analysis

The normality of data distribution was tested using Q-Q plots and the D'Agostino-Pearson test [19]. As most data did not follow a normal distribution in eGDR subgroups, summaries are presented as medians and interquartile ranges (IQR). Comparisons between eGDR groups were made using the Mann-Whitney test for unpaired data. Categorical data are presented as numbers (percentages), while numerical data are presented as medians (lower to upper quartiles) and compared using the Fisher exact test. All tests were two-sided, and the p-value was set at < 0.05 as statistically significant. All statistical analyses were made with the custom code of the R-programming language (version 3.6.1.; Vienna, R Project).

We performed a logistic regression analysis to identify factors associated with cardiac remodeling. The dependent variable was defined as increased IVST (>1 cm) [20]. The multivariable model included age, sex (coded as 1 for men), and BMI—well-established factors related to cardiac size—along with the presence of higher IR risk (defined as eGDR at or below the median value) [21].

Results

Comparison of clinical characteristics

The clinical characteristics of both groups are presented in **Table 1**. We investigated 84 adults with a median age of 40.5 (34.0–51.0) years and a diabetes duration of 21.0 (15.5–27.0) years. Fifty-two (61.9%) of them were men. Median eGDR was 7.4 (5.1–9.5). Most participants with eGDR at or below the median value had hypertension and more commonly presented with diabetic retinopathy and kidney disease. They also required metformin, statins, beta-blockers, and ACEI/ARB more frequently but were less often on insulin pumps. These individuals were older (median age difference of 4.5 years), although the duration of T1DM was comparable between both groups. Their cardiometabolic and renal profiles were

worse, with higher systolic and diastolic blood pressure (SBP, DBP), BMI, WHR, HbA1c, white blood cell count (WBC), triglycerides (TGA), and poorer kidney function.

Comparison of cardiac remodeling

Table 2 summarizes the echocardiographic findings. People with eGDR below or equal to the median had significantly more dilated end-di-

Table 1. Comparison of clinical characteristics between individuals with T1DM and eGDR > (IR less probable) or ≤ the median (IR more probable).

Clinical feature or parameter	eGDR > the median median (IQR) n = 42 (50%)	eGDR ≤ the median median (IQR) n = 42 (50%)	p-value
	Categorical data N (% of a group)		
Men	23 (54.8)	29 (69.0)	0.18
Current smoker	6 (14.3)	9 (21.4)	0.39
Hypertension	**1 (2.4)**	**38 (90.5)**	**<0.01**
Peripheral diabetic neuropathy	7 (16.7)	12 (28.6)	0.19
Diabetic retinopathy	**9 (21.4)**	**24 (57.1)**	**<0.01**
Diabetic kidney disease	**2 (4.8)**	**10 (23.8)**	**0.01**
Diabetic foot	1 (2.4)	2 (4.8)	0.56
On insulin pump	**12 (28.6)**	**2 (4.8)**	**<0.01**
Metformin	**4 (9.5)**	**12 (28.6)**	**0.02**
Statin	**3 (7.1)**	**11 (26.2)**	**0.01**
Beta-blocker	**2 (4.8)**	**12 (28.6)**	**<0.01**
ACEI/ARB	**2 (4.8)**	**28 (66.7)**	**<0.01**
	Continuous data median (IQR)		
Age [years]	**38.5 (31–51)**	**43 (38–51)**	**0.01**
T1DM duration [years]	19.5 (15–26)	22.5 (17–28)	0.39
SBP [mmHg]	**127 (117.5–136)**	**130 (125–142)**	**0.01**
DBP [mmHg]	**79.5 (75–86)**	**84.5 (77.5–92)**	**0.04**
WHR	**0.8 (0.8–0.9)**	**0.9 (0.9–1)**	**<0.01**
BMI [kg/m^2]	**25.2 (23.5–27.5)**	**28.2 (24.7–31.8)**	**0.01**
HbA1c [%]	**7.4 (7–8.8)**	**8.1 (7.4–9.2)**	**0.01**
White Blood Cells [G/l]	**6.24 (5.22–7.01)**	**7 (6.24–8.03)**	**0.01**
Red Blood Cells [T/l]	4.94 (4.59–5.18)	4.82 (4.68–5.18)	0.86
Hemoglobin [g/l]	14.4 (13.6–15.1)	14.75 (13.6–15.3)	0.8
Sodium [mmol/l]	139 (138–141)	139 (138–140)	0.91
Potassium [mmol/l]	4.15 (4–4.31)	4.31 (4.05–4.65)	0.07
Total cholesterol [mg/dl]	185.5 (171–202)	190 (165–233)	0.5
LDL-C [mg/dl]	100.5 (86–122)	103 (82–132)	0.63
HDL-C [mg/dl]	63.5 (54–71)	55.5 (50–72)	0.15
TGA [mg/dl]	**85.5 (59–102)**	**108.5 (89–150)**	**<0.01**
Non-HDL-C [mg/dl]	119.5 (106–144)	134 (100–159)	0.27
TGA/HDL	**1.30 (0.84–1.85)**	**1.92 (1.08–2.88)**	**<0.01**
ALT [UI/l]	17 (13–20)	18 (14–32)	0.16
AST [UI/l]	18 (16–21)	18.5 (16–27)	0.16
Creatinine [mg/dl]	**0.83 (0.75–0.91)**	**0.91 (0.78–1)**	**0.03**
eGFR [ml/min/1.73m^2]	**104.3 (90–113. 8)**	**92.2 (87.6–103.9)**	**0.01**
ACR [mg/g]	**4 (3–6)**	**6 (3–20.76)**	**0.04**
CRP [mg/l]	1.05 (0.77–1.82)	1.38 (0.73–2.22)	0.2
TSH [µIU/ml]	2.01 (1.15–2.73)	1.68 (1.18–2.3)	0.23

ACR – Albumin to Creatinine Ratio. ALT – Alanine Transaminase. AST – Aspartate Transaminase. BMI – Body Mass Index. HbA1c – Glycated Hemoglobin. HDL-C – High-Density Lipoprotein cholesterol. LDL-C – Low-Density Lipoprotein cholesterol. WHR – Waist-to-Hip Ratio. MCHC – Mean Corpuscular Hemoglobin Concentration. TGA – triglycerides. TSH – Thyroid-stimulating Hormone. eGDR - Estimated Glucose Disposal Rate. SD - Standard Deviation. eGFR - estimated glomerular filtration rate. SBP - Systolic Blood Pressure. DBP - Diastolic Blood Pressure, ACEI angiotensin-converting enzyme inhibitors, ARB - AT1 receptor blockers; p < 0.05 bolded.

Table 2. Comparison of echocardiographic measurements between individuals with T1DM and eGDR > (IR less probable) or ≤ the median (IR more probable).

Echocardiographic measurement	eGDR > the median median (IQR) n = 42 (50%)	eGDR ≤ the median median (IQR) n = 42 (50%)	p-value
LVEDD [cm]	**4.5 (4.3–4.8)**	**4.75 (4.5–5)**	**0.01**
RVEDD [cm]	**2.7 (2.5–2.8)**	**2.8 (2.6–2.9)**	**0.02**
IVST [cm]	**0.9 (0.8–1)**	**1.1 (1–1.18)**	**<0.01**
LAD [cm]	**3.5 (3.2–3.7)**	**3.8 (3.5–3.9)**	**<0.01**
EF [%]	55 (55–60)	55 (55–55)	0.9

LVEDD – left ventricular end-diastolic diameter. LAD – left atrial diamater. RVEDD – right ventricular end-diastolic diameter, EF- ejection fraction, IVST - interventricular septal thickness; p < 0.05 bolded.

Table 3. Multivariable logistic regression model for the presence of IVS hypertrophy defined as IVST > 1 cm (codes as 1) vs IVST ≤ to 1 cm (coded as 0).

Variable	OR [95% CI]	p
Age [years]	1.01 [95% CI: 0.96–1.07]	0.65
Sex (man)	**5.22 [95% CI: 1.47–18.46]**	**0.01**
BMI [kg/m^2]	1.09 [95% CI: 0.96–1.23]	0.17
eGDR ≤median	**7.55 [95% CI: 2.24–25.50]**	**<0.01**

CI – Confidence Interval; OR – Odds Ratio; eGDR - estimated Glucose Disposal Rate, IVST - interventricular septal thickness; p < 0.05 bolded.

astolic left ventricle and right ventricle diameters, end-systolic left atrial diameter, and thicker intraventricular septum at the end of diastole. Although statistically significant, the differences between medians were of low clinical significance, ranging from 0.1 cm for RVEDD to 0.25 cm for LVEDD. Notably, left ventricular EF was comparable between both groups.

The multivariable logistic regression model revealed that thicker interventricular septum (IVS) was positively associated with eGDR ≤ median adjusted for BMI, age, and sex (see **Table 3**). Increased IVST was more common in those with eGDR at or below the median value (more insulin resistant) and in men. Therefore, we included separate analyses of cardiac dimensions for the women and men in the supplementary material (**Supplementary Tables 1** and **2**).

In univariable logistic regression analysis, eGDR at or below the median was positively related to LVEDD (OR 3.40 [95% CI: 1.04–11.14]; p = 0.04) and LAD (OR 4.23 [95% CI: 1.33–13.42]; p = 0.01). However, the association between increased LAD and eGDR at or below the median became nonsignificant after adjusting for sex, age, and BMI (p = 0.64). No participants showed LV dilation defined as LVEDD above 5.9 cm in men or 5.2 cm in women [20].

Discussion

Our study demonstrates that increased risk of insulin resistance (IR), assessed through eGDR, is associated with a worse cardiometabolic and renal profile, more diabetic complications, and more complex pharmacological treatment. Individuals with T1DM and a higher probability of IR are usually overweight. They also tend to have more dilated left and right ventricles, left atrium, and IVS hypertrophy. Increased IVST occurred more often in less insulin-sensitive T1DM individuals, regardless of BMI, age, or sex. These findings indicate that people with T1DM and a higher likelihood of IR exhibit at least mild but adverse cardiac remodeling.

The measurements of left ventricular diameters are related to subclinical heart failure [22]. As assessed by HOMA-IR, insulin resistance is associated with subclinical left ventricular dysfunction in the general population [23]. Compared to healthy controls, increased left ventricular diameter, mass, and left atrial volume are commonly observed in individuals with metabolic syndrome [24]. Additionally, left ventricular diameter has been linked to a higher risk of sudden cardiac death, independent of ejection fraction [25]. These findings are consistent with previous research on

T2DM, which also associates IR with heart failure [10,26]. In T2DM, BMI has been positively associated with left ventricular dimensions, and left ventricular diastolic diameter has been identified as a standalone indicator of mortality risk [27,28]. In our study, men exhibited higher odds of IVS hypertrophy. This finding is in agreement with other studies demonstrating that women have lower left ventricular wall thickness than men [29].

Pathophysiology

The impact of IR on cardiac hypertrophy in T1DM remains less explored, though mechanisms in T2DM provide some insight. Chronic hyperinsulinemia, a hallmark of IR, contributes to increased left ventricular diameters in T2DM [30]. IR impairs the PI3K/Akt signaling pathway, which normally promotes vasodilation and reduces vascular resistance, while leaving the MAPK pathway, which drives cell proliferation and endothelin-1 secretion, largely intact [31,32]. Increased endothelin-1 concentration and sympathetic tone lead to hypertension and cardiac hypertrophy [33]. Over time, the proliferative effects of insulin on vascular smooth muscle cells cause an increase in left ventricular mass and concentric remodeling, potentially going unnoticed until more severe cardiovascular issues arise [30,34]. In people with cardiac hypertrophy due to aortic stenosis, myocardial glucose uptake was lower during high insulin conditions, indicating insulin resistance. This resistance is associated with changes in glucose transporters, specifically a decreased GLUT-4/GLUT-1 ratio [35].

In our study, WBC count was higher in participants with a higher probability of IR and mild, although adverse, cardiac remodeling. This finding aligns with previous research, such as Shi et al., which found a positive relationship between WBC count and left ventricular mass index in hypertensives [36]. Leukocytes play a significant role in diabetic cardiomyopathy in T2DM, with disturbances in metabolic and inflammatory pathways due to pro-inflammatory cytokines, glucose metabolites, and reactive oxygen species during hyperglycemia. This increases leukocyte activation and cardiac inflammation, with neutrophils and macrophages contributing to sustained inflammation and fibrosis [37]. However, whether WBC contributes to cardiac remodeling in people with T1DM is uncertain; a more common finding and potential correlation do not necessarily translate into causation.

Few studies have investigated echocardiographic changes in diabetic cardiomyopathy in T1DM. Weber et al. reported that young adults with T1DM had significantly higher interventricular septal thickness and reduced diastolic parameters than non-diabetic controls [11]. Additionally, echocardiographic parameters such as left ventricular EF <45%, impaired global longitudinal strain, and diastolic mitral early velocity (E)/early diastolic tissue Doppler velocity (e') were predictive of major adverse cardiovascular events (MACE) in people with T1DM without heart disease over a 7.5-year follow-up [38]. Lassen et al. suggested that E/e' and global longitudinal strain (GLS) provide better prognostic value in T1DM women than men [39]. In another study, BMI was positively associated with left atrial volume, left ventricular mass, and transmitral Doppler ratio E/A in T1DM individuals without previous heart disease or hypertension [12]. In a cohort of 20,985 people with T1DM, the incidence of HF was positively associated with HbA1c, age, diabetes duration, BMI, smoking, SBP, and negatively with HDL-C [40]. However, Hjortkjær et al. found that long-term T1DM was associated with smaller left ventricular mass and volumes [41].

Insulin resistance is a well-known risk factor for heart failure and death in people with T2DM [42]. In T1DM individuals with IR, there is also some evidence of worse cardiovascular outcomes. An analysis of women with T1DM aged 65 to 74 showed that the presence of IR was accompanied by a more common history of myocardial infarction [43].

Study limitations

There are several limitations to this study. First, instead of using the complicated and time-consuming hyperinsulinemic-euglycemic clamp method, we used the indirect eGDR method. However, this approach is increasingly common in daily clinical settings. Another limitation is the cross-sectional nature of the study, which shows that some findings coexist and may be associated but do not establish causation. A prospective

study with long-term follow-up, more detailed cardiovascular evaluation, insulin resistance measurements or animal models might provide clearer answers.

Additionally, this is a single-center study performed on a group of T1DM patients under the constant care of a regional reference center for diabetes. Although the median duration of T1DM was 15 years, these people generally had well-preserved kidney function, lipid profiles, and SBP within target ranges. At least half of them had normal BMI. Even if cardiac remodeling was present, it was mild. In other settings, people with T1DM of similar duration might be in more severe clinical conditions. Although insulin pump treatment is the gold standard in T1DM, it is still not used by all individuals, even in our group. The mode of insulin treatment might also impact the rate of IR, clinical profile, and cardiac remodeling. Finally, the medium sample size and single-center nature of the study may limit the generalizability of our findings.

Future research should aim to include larger and more diverse populations, control groups, comprehensive cardiac assessments, and use gold-standard methods for insulin resistance evaluation to enhance the validity and applicability of the findings.

Perspectives

Among adults with T1DM, increased risk of IR, as estimated by eGDR, is positively associated with adverse cardiac remodeling. It remains unclear whether insulin itself promotes IVS hypertrophy or if other factors contributing to IR are responsible for the adverse cardiac remodeling.

These findings highlight the importance of early identification and management of insulin resistance in T1DM to mitigate cardiac complications potentially. Future studies should focus on longitudinal assessments to better understand the progression of cardiac remodeling in this population and explore targeted interventions to improve cardiovascular outcomes.

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This paper is dedicated to Pawel Lachowski, who passed away during the preparation of this article.

Authors contribution

Michal Kulecki wrote the main manuscript text. He was also responsible for the study's conceptualization, methodology, formal analysis, investigation, and methodology. Dariusz Naskret was responsible for project administration, conceptualization, investigation, and Resources. Mikolaj Kaminski was responsible for formal analysis, supervision, investigation, and resources. Dominika Kasprzak was responsible for conceptualization, investigation, and resources. Pawel Lachowski, Daria Klause, Maria Kozlowska, and Justyna Flotynska were responsible for the investigation and resources. Dariusz Naskret, Aleksandra Uruska, and Dorota Zozulinska-Ziolkiewicz supervised the project. All authors reviewed the manuscript.

Data availability

The data supporting this study's findings are available from the corresponding author upon reasonable request.

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Conflict of interest statement

The authors declare no conflict of interest.

References

1. Araszkiewicz A, Bandurska-Stankiewicz E, Borys S, Budzyński A, Cyganek K, Cypryk K et al. 2022 Guidelines on the management of patients with diabetes. A position of Diabetes Poland. Current Topics in Diabetes. 2022;2(1):1-130. doi: 10.5114/ctd/146259.
2. Mao Y, Zhong W. Changes of insulin resistance status and development of complications in type 1 diabetes mellitus: Analysis of DCCT/EDIC study. Diabetes Res Clin Pract. 2022 Feb;184:109211. doi: 10.1016/j.diabres.2022.109211.
3. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol. 1979 Sep;237(3):E214-23. doi: 10.1152/ajpendo.1979.237.3.E214.
4. Epstein EJ, Osman JL, Cohen HW, Rajpathak SN, Lewis O, Crandall JP. Use of the estimated glucose disposal rate as a measure of insulin resistance in an urban multiethnic population with type 1 diabetes. Diabetes Care. 2013 Aug;36(8):2280-5. doi: 10.2337/dc12-1693.
5. Šimonienė D, Platūkiene A, Prakapienė E, Radzevičienė L, Veličkiene D. Insulin Resistance in Type 1 Diabetes Mellitus and Its Association with Patient's Micro- and Macrovascular Complications, Sex Hormones, and Other Clinical Data. Diabetes Ther. 2020 Jan;11(1):161-174. doi: 10.1007/s13300-019-00729-5.
6. Garofolo M, Gualdani E, Scarale MG, Bianchi C, Aragona M, Campi F, Lucchesi D, Daniele G, Miccoli R, Francesconi P, Del Prato S, Penno G. Insulin Resistance and Risk of Major Vascular Events and All-Cause Mortality in Type 1 Diabetes: A 10-Year Follow-up Study. Diabetes Care. 2020 Oct;43(10):e139-e141. doi: 10.2337/dc20-0433.

7. Avogaro A, Azzolina D, Fadini GP, Baldi I. Incidence of heart failure in patients with type 1 diabetes: a systematic review of observational studies. J Endocrinol Invest. 2021 Apr;44(4):745-753. doi: 10.1007/s40618-020-01368-5.
8. Katogiannis K, Vlastos D, Kousathana F, Thymis J, Kountouri A, Korakas E, Plotas P, Papadopoulos K, Ikonomidis I, Lambadiari V. Echocardiography, an Indispensable Tool for the Management of Diabetics, with or without Coronary Artery Disease, in Clinical Practice. Medicina (Kaunas). 2020 Dec 18;56(12):709. doi: 10.3390/medicina56120709.
9. Jia G, Whaley-Connell A, Sowers JR. Diabetic cardiomyopathy: a hyperglycaemia- and insulin-resistance-induced heart disease. Diabetologia. 2018 Jan;61(1):21-28. doi: 10.1007/s00125-017-4390-4.
10. Liu JJ, Liu S, Ang KA, Lim SC. Estimated glucose disposal rate, a novel biomarker for insulin sensitivity, predicts risk for incident heart failure in patients with type 2 diabetes. European Heart Journal. 2023;44:ehac779.035. doi: 10.1093/eurheartj/ehac779.035.
11. Weber TR, da Silva RL, Cossul S, Alves MSL, Lee SVS, Marques JLB. The use of echocardiography in type 1 diabetes. Rev Port Cardiol (Engl Ed). 2021 Jul 19:S0870-2551(21)00249-3. English, Portuguese. doi: 10.1016/j.repc.2020.11.012.
12. Šuran D, Kanič V, Naji F, Krajnc I, Čokolič M, Zemljič E, Sinkovič A. Predictors of early cardiac changes in patients with type 1 diabetes mellitus: An echocardiography-based study. Bosn J Basic Med Sci. 2019 Jun 18;19(4):384-391. doi: 10.17305/bjbms.2019.4250.
13. Sawicka-Gutaj N. Publication ethics of human studies in the light of the Declaration of Helsinki – a mini-review. Journal of Medical Science. 2022;91(2):e700. doi: 10.20883/medical.e700.
14. Kulecki M, Naskret D, Kaminski M, Kasprzak D, Lachowski P, Klause D, Kozlowska M, Flotynska J, Uruska A, Zozulinska-Ziolkiewicz D. Association between central non-dipping pattern and platelet morphology in adults with type 1 diabetes without cardiovascular disease: a cross-sectional study. Sci Rep. 2021 Jul 29;11(1):15416. doi: 10.1038/s41598-021-94414-y.
15. Kulecki M, Naskret D, Kaminski M, Kasprzak D, Lachowski P, Klause D, Kozlowska M, Flotynska J, Uruska A, Zozulinska-Ziolkiewicz D. Arterial stiffness and the non-dipping pattern in type 1 diabetes males with and without erectile dysfunction. Sci Rep. 2023 May 4;13(1):7265. doi: 10.1038/s41598-023-33315-8.
16. Kamiński M, Kulecki M, Lachowski P, Kasprzak D, Kulczycka A, Kozłowska M, Klause D, Uruska A, Michalski M, Zozulińska-Ziółkiewicz D. Erectile Dysfunction in Individuals with Type 1 Diabetes is Associated with Long-term Metabolic Control and Diabetic Complications: A Cross-Sectional Study. Int J Angiol. 2022 Jan 13;31(2):97-106. doi: 10.1055/s-0041-1735209.
17. Galderisi M, Cosyns B, Edvardsen T, Cardim N, Delgado V, Di Salvo G, Donal E, Sade LE, Ernande L, Garbi M, Grapsa J, Hagendorff A, Kamp O, Magne J, Santoro C, Stefanidis A, Lancellotti P, Popescu B, Habib G; 2016–2018 EACVI Scientific Documents Committee; 2016–2018 EACVI Scientific Documents Committee. Standardization of adult transthoracic echocardiography reporting in agreement with recent chamber quantification, diastolic function, and heart valve disease recommendations: an expert consensus document of the European Association of Cardiovascular Imaging. Eur Heart J Cardiovasc Imaging. 2017 Dec 1;18(12):1301-1310. doi: 10.1093/ehjci/jex244.
18. Lipiec P, Bąk J, Braksator W, Fijałkowski M, Gackowski A, Gąsior Z, Kasprzak JD, Klisiewicz A, Kowalski M, Kukulski T, Mizia-Stec K, Płońska-Gościniak E, Pruszczyk P, Sobkowicz B, Szymański P, Szyszka A, Trojnarska O, Wysokiński A, Hoffman P. Transthoracic echocardiography in adults — guidelines of the Working Group on Echocardiography of the Polish Cardiac Society [Transthoracic echocardiography in adults - guidelines of the Working Group on Echocardiography of the Polish Cardiac Society]. Kardiol Pol. 2018;76(2):488-493. Polish. doi: 10.5603/KP.2018.0051.
19. Guzik P, Więckowska B. Data distribution analysis – a preliminary approach to quantitative data in biomedical research. Journal of Medical Science. 2023;92:e869–e869. doi: 10.20883/medical.e869.
20. Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, Flachskampf FA, Foster E, Goldstein SA, Kuznetsova T, Lancellotti P, Muraru D, Picard MH, Rietzschel ER, Rudski L, Spencer KT, Tsang W, Voigt JU. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. J Am Soc Echocardiogr. 2015 Jan;28(1):1-39.e14. doi: 10.1016/j.echo.2014.10.003.
21. Pfaffenberger S, Bartko P, Graf A, Pernicka E, Babayev J, Lolic E, Bonderman D, Baumgartner H, Maurer G, Mascherbauer J. Size matters! Impact of age, sex, height, and weight on the normal heart size. Circ Cardiovasc Imaging. 2013 Nov;6(6):1073-9. doi: 10.1161/CIRCIMAGING.113.000690.
22. Zhu N, Chen H, Zhao X, Ye F, Jiang W, Wang Y. Left atrial diameter in heart failure with left ventricular preserved, mid-range, and reduced ejection fraction. Medicine (Baltimore). 2019 Nov;98(48):e18146. doi: 10.1097/MD.0000000000018146.
23. Hirose K, Nakanishi K, Daimon M, Sawada N, Yoshida Y, Iwama K, Yamamoto Y, Ishiwata J, Hirokawa M, Koyama K, Nakao T, Morita H, Di Tullio MR, Homma S, Komuro I. Impact of insulin resistance on subclinical left ventricular dysfunction in normal weight and overweight/obese japanese subjects in a general community. Cardiovasc Diabetol. 2021 Jan 21;20(1):22. doi: 10.1186/s12933-020-01201-6.
24. Sakthivadivel V, Chinnathambi C, Selvaraju D, Gaur A, Bakthavatchalam R, Srivijayan A, Sundaramurthy R, Tadi LJ, John NA. Metabolic syndrome - cardiac structure and functional analysis by echocardiography; a cross sectional comparative study. Horm Mol Biol Clin Investig. 2023 Mar 2;44(3):305-310. doi: 10.1515/hmbci-2022-0066.
25. Left Ventricular Diameter and Risk Stratification for Sudden Cardiac Death – Journal of the Ameri-

can Heart Association. https://www.ahajournals.org/doi/10.1161/JAHA.114.001193. Accessed 15 Aug 2024.
26. Otowa-Suematsu N, Sakaguchi K, Kaneko A, Ito J, Morita Y, Miura H, Yamada T, So A, Komada H, Okada Y, Hirota Y, Tamori Y, Ogawa W. Relation of cardiac function to insulin resistance as evaluated by hyperinsulinemic-euglycemic clamp analysis in individuals with type 2 diabetes. J Diabetes Investig. 2021 Dec;12(12):2197-2202. doi: 10.1111/jdi.13608.
27. Echeverría LE, Saldarriaga C, Campbell-Quintero S, Morales-Rodríguez LN, López-Ponce de León JD, Buitrago AF, Martínez-Carreño E, Sandoval-Luna JA, Llamas A, Moreno-Silgado GA, Vanegas-Eljach J, Murillo-Benítez NE, Gómez-Paláu R, Rivera-Toquica AA, Gómez-Mesa JE, Research Group R. Diabetes mellitus in patients with heart failure and effect modification of risk factors for short-term mortality: An observational study from the Registro Colombiano de Falla Cardíaca (RECOLFACA). Biomedica. 2024 May 31;44(Sp. 1):182-197. doi: 10.7705/biomedica.6951.
28. Zhang J, Yang Z, Fan X, Fei Q, Xi Y. Positive Correlation between BMI and Left Ventricle and Atrium Inside Diameter Size in Chinese Type 2 Diabetes Patients with Left Ventricular and Atrial Enlargement. Rev Cardiovasc Med. 2024 Jun 4;25(6):207. doi: 10.31083/j.rcm2506207.
29. Khoury S, Zornitzki L, Laufer-Perl M, Bhatia RT, Marwaha S, Tome M, Granot Y, Gvili Perelman M, Avivi I, Shacham Y, Szekely Y, Banai S, Hochstadt A, Flint N, Topilsky Y. Sex-related differences in the association between septal wall thickness and survival. Int J Cardiol Heart Vasc. 2024 May 27;53:101427. doi: 10.1016/j.ijcha.2024.101427.
30. Fazio S, Mercurio V, Tibullo L, Fazio V, Affuso F. Insulin resistance/hyperinsulinemia: an important cardiovascular risk factor that has long been underestimated. Front Cardiovasc Med. 2024 Mar 13;11:1380506. doi: 10.3389/fcvm.2024.1380506.
31. Abel ED. Insulin signaling in the heart. Am J Physiol Endocrinol Metab. 2021 Jul 1;321(1):E130-E145. doi: 10.1152/ajpendo.00158.2021.
32. Cai W, Sakaguchi M, Kleinridders A, Gonzalez-Del Pino G, Dreyfuss JM, O'Neill BT, Ramirez AK, Pan H, Winnay JN, Boucher J, Eck MJ, Kahn CR. Domain-dependent effects of insulin and IGF-1 receptors on signalling and gene expression. Nat Commun. 2017 Mar 27;8:14892. doi: 10.1038/ncomms14892.
33. Madonna R, Pandolfi A, Massaro M, Consoli A, De Caterina R. Insulin enhances vascular cell adhesion molecule-1 expression in human cultured endothelial cells through a pro-atherogenic pathway mediated by p38 mitogen-activated protein-kinase. Diabetologia. 2004 Mar;47(3):532-536. doi: 10.1007/s00125-004-1330-x.
34. Stout RW. Insulin as a mitogenic factor: role in the pathogenesis of cardiovascular disease. Am J Med. 1991 Feb 21;90(2A):62S-65S. doi: 10.1016/0002-9343(91)90041-u.
35. Paternostro G, Pagano D, Gnecchi-Ruscone T, Bonser RS, Camici PG. Insulin resistance in patients with cardiac hypertrophy. Cardiovasc Res. 1999 Apr;42(1):246-53. doi: 10.1016/s0008-6363(98)00233-8.
36. Shi H, Chu H, Lv Z, Qi G, Guo J, Fu W, Wang X, Guo X, Ge J, Yin C. Association of white blood cell counts with left ventricular mass index in hypertensive patients undergoing anti-hypertensive drug therapy. Exp Ther Med. 2017 Apr;13(4):1566-1571. doi: 10.3892/etm.2017.4119.
37. Bajpai A, Tilley DG. The Role of Leukocytes in Diabetic Cardiomyopathy. Front Physiol. 2018 Nov 1;9:1547. doi: 10.3389/fphys.2018.01547.
38. Jensen MT, Sogaard P, Gustafsson I, Bech J, Hansen TF, Almdal T, Theilade S, Biering-Sørensen T, Jørgensen PG, Galatius S, Andersen HU, Rossing P. Echocardiography improves prediction of major adverse cardiovascular events in a population with type 1 diabetes and without known heart disease: the Thousand & 1 Study. Diabetologia. 2019 Dec;62(12):2354-2364. doi: 10.1007/s00125-019-05009-2.
39. Lassen MCH, Biering-Sørensen T, Jørgensen PG, Andersen HU, Rossing P, Jensen MT. Sex differences in the association between myocardial function and prognosis in type 1 diabetes without known heart disease: the Thousand & 1 Study. Eur Heart J Cardiovasc Imaging. 2021 Aug 14;22(9):1017-1025. doi: 10.1093/ehjci/jeaa227.
40. Lind M, Bounias I, Olsson M, Gudbjörnsdottir S, Svensson AM, Rosengren A. Glycaemic control and incidence of heart failure in 20,985 patients with type 1 diabetes: an observational study. Lancet. 2011 Jul 9;378(9786):140-6. doi: 10.1016/S0140-6736(11)60471-6.
41. Hjortkjær HØ, Jensen T, Hilsted J, Mogensen UM, Corinth H, Rossing P, Sigvardsen PE, Fuchs A, Kühl JT, Nordestgaard BG, Køber L, Kofoed KF. Cardiac ventricular sizes are reduced in patients with long-term, normoalbuminuric type 1 diabetes compared to the non-diabetic background population. Diab Vasc Dis Res. 2019 May;16(3):289-296. doi: 10.1177/1479164118819961.
42. Wamil M, Coleman RL, Adler AI, McMurray JJV, Holman RR. Increased Risk of Incident Heart Failure and Death Is Associated With Insulin Resistance in People With Newly Diagnosed Type 2 Diabetes: UKPDS 89. Diabetes Care. 2021 Aug;44(8):1877-1884. doi: 10.2337/dc21-0429.
43. Dereziński T, Zozulińska-Ziółkiewicz D, Uruska A, Dąbrowski M. Visceral adiposity index as a useful tool for the assessment of cardiometabolic disease risk in women aged 65 to 74. Diabetes Metab Res Rev. 2018 Nov;34(8):e3052. doi: 10.1002/dmrr.3052.

Supplementary Table 1. Comparison of echocardiographic measurements between clinical characteristics between men with T1DM and eGDR > or ≤ the median.

Echocardiographic measurement	eGDR > the median median (IQR)	eGDR ≤ the median median (IQR)	p-value
LVEDD [cm]	**4.7 (4.5–4.9)**	**5.0 (4.8–5)**	**0.01**
RVEDD [cm]	2.7 (2.6–2.9)	2.8 (2.7–2.9)	0.33
IVST [cm]	**1.0 (0.9–1)**	**1.1 (1.1–1.2)**	**<0.01**
LAD [cm]	**3.6 (3.4–3.8)**	**3.9 (3.8–4.0)**	**<0.01**
EF [%]	55 (55–60)	55 (55–55)	0.50

LVEDD – left ventricular end-diastolic diameter. LAD – left atrial diamater. RVEDD – right ventricular end-diastolic diameter, EF- ejection fraction, IVST - interventricular septal thickness; p < 0.05 bolded.

Supplementary Table 2. Comparison of echocardiographic measurements between clinical characteristics between women with T1DM and eGDR > or ≤ the median.

Echocardiographic measurement	eGDR > the median median (IQR)	eGDR ≤ the median median (IQR)	p-value
LVEDD [cm]	4.3 (4.2–4.5)	4.5 (4.2–4.6)	0.62
RVEDD [cm]	2.6 (2.5–2.7)	2.7 (2.6–3.2)	0.07
IVST [cm]	0.9 (0.8–0.9)	1.0 (0.8–1.1)	0.21
LAD [cm]	3.2 (3.0–3.6)	3.5 (3.1–3.7)	0.27
EF [%]	55 (55–60)	55 (55–55)	0.93

LVEDD – left ventricular end-diastolic diameter. LAD – left atrial diamater. RVEDD – right ventricular end-diastolic diameter, EF- ejection fraction, IVST - interventricular septal thickness.

ORIGINAL PAPER

JMS Journal of Medical Science

Effect of flipped classroom on student perceptions, academic scores and study practices

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ABSTRACT

Aim. To evaluate the educational impact of two flipped classroom models.
Material and methods. Traditional lectures were followed by Flipped classroom (FC) with face to face(F2F) session (cohort of 2019-20) and online (cohort 2020-21). Students' perceptions were collected by questionnaire after lectures and FC. Historic academic scores on selected topic were compared scores after introduction of FC. The learning management system(LMS) was used to monitor student usage of learning resources.
Results. Students perceptions for cohort 2019-20 were significantly higher($p>0.05$) for FC(F2F) compared to lecture. Students' perceptions for cohort 2020-21 were also significantly higher for FC(online) compared to online lecture. Academic scores did not show significant difference between lecture and FC. Increased hits on LMS for online resources were associated with FC or summative assessment.
Conclusions. Students were overwhelmingly positive on FC for both: with F2F and online , and academic scores were similar to lecture. FC can possibly improve student study habits but needs further research.

Introduction

A lecture, as a teaching method, inherently suffers from being able to focus largely on the lower levels of cognition, leaving little or no time to address the higher levels [1]. A passive transfer of knowledge and an instructor centric relationship is deemed to be unsuitable for the current generation of learners [2]. The flipped classroom (FC) has garnered considerable attention as an alternative to the traditional lecture. The FC is a variant of blended learning [3,4]. Broadly, FC model involves provision of learning resources online prior to the face to face (F2F) session. The resources are utilized by students to acquire lower- level cognitive knowledge, creating sufficient time in the formal

F2F session to address higher levels, by engaging in active learning strategies [5].

The FC model has been extensively researched, particularly in higher education. Literature in health sciences has also been growing and systematic reviews or meta-analyses on certain aspects of the flipped classroom [6,7] have been conducted. In dental education, studies have reported a positive effect of FC on student perception [8]. Nevertheless, the influence of FC on academic scores has yielded mixed results [9] and literature describing its effect on behavioral change is limited.

The advent of COVID-19 pandemic has resulted in most dental schools conducting the didactic sessions online [10] and "ensuring the continuity and quality of dental education" is a challenge [11] that needs rapid adaptation of existing teaching strategies to engage students effectively. Under the circumstances, all didactic activities were shifted to online mode and the F2F session in the FC was replaced with an online one.

Thus, the aim of the study was; to evaluate the educational impact of two FC models.

Research questions

1. What is the effect of the FC models (F2F and online) on student perceptions.
2. What is the effect of flipped classroom on the academic scores (in didactic component).
3. What is the effect of FC on study habits of students?

Materials and methods

The FC was introduced to final year students of dentistry program in the course: Comprehensive orthodontics and pediatric dentistry. **Figure 1** is describing flow chart showing the key considerations and activities during implementation of the FC (conventional/online).

The cohort for 2019–20 had 95 students. The FC was implemented after approval from the institutional ethics committee and research center board coupled with support from the IT department. A workshop was conducted by the institutional medical education unit to orient faculty and help design the FC model. Extensive review of literature was performed to establish a resource for reference during the implementation.[12] An orientation class was conducted for the students prior to the FC wherein they were introduced to the concept of FC and the necessity of varied methods of teaching was explained. The first 3 topics of the course were conducted by traditional lecture (50 minutes each) and at the end of these lectures, students were asked to respond to a pretested questionnaire on the traditional lecture. Topics "pulp therapy in primary and young permanent dentition" (10% of the didactic component of the course) were selected to be conducted as a FC. Traditionally, these were conducted as 2 separate lectures of 50 minutes each.

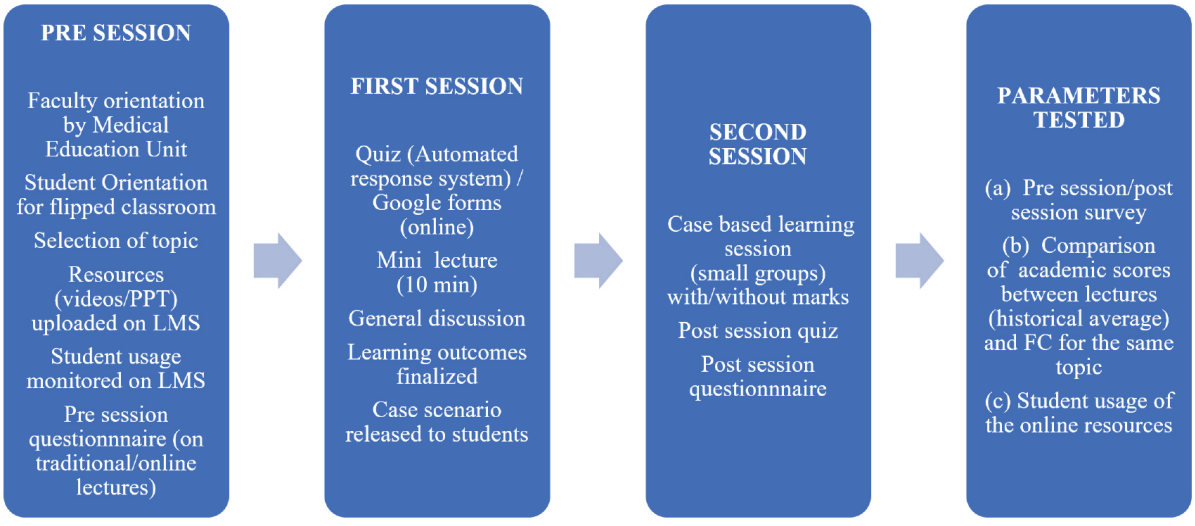

Figure 1. Flow chart showing the key considerations and activities during implementation of the FC (conventional/online).

FC (F2F)

Resources including: video recorded lectures(VRL), power-point(PPT) presentations of lectures, and links to external open access sources such American Academy of Pediatric Dentistry (AAPD) guidelines, were uploaded on the Learning Management System(LMS), Moodle, 1 week before the session.

The flipped classroom (F2F) consisted of two sessions. In the first session(40 minutes), students were initially exposed to a quiz including questions on both the topics that were uploaded one week before the lecture. The quiz was conducted with POWER VOTE™ (Automated response system) and the correct answers were displayed in real time. Results were used to give feedback and clarify student's misconceptions. This was followed by a mini lecture of 10 minutes and a general discussion on questions with students. A case scenario designed by faculty were released to the students and learning outcomes were established for the next session scheduled one week later.

In the second session, students were divided into smaller groups (7 or less) for a case-based learning session(CBL) for a duration of 80 minutes. The scenario consisted of a brief introduction to the problem, followed by sequential disclosure of history, findings of clinical examination and investigations. CBL sessions included role play by faculty (for parts of history taking) and summary (5 minutes at end of the session). Students were assessed with case- based assessment sheet using analytic rubrics. All other activities in the course were kept the same as previous years. The summative assessment was a mix of MCQ's and short answer questions(SAQ). The selected topic had a total of 10 MCQ's and 1 SAQ included in the assessments. The questions were framed by a single faculty for all the years and the assessment was verified by a committee.

FC (online)

In the years 2020–21, all the lectures were conducted online due to the COVID-19 pandemic. The F2F session was substituted with an online one conducted on ZOOM. Online quiz was conducted with Google forms and case- based learning session was conducted using breakout rooms. Additionally, students were asked to prepare and submit a concept map (1 per group) for the case scenario. The session was also assigned marks (2.5% out of overall 100) and students were informed the same during orientation sessions.

Students of both years responded to a pretested post session questionnaire on their perceptions of the FC. The first seven questions of both pre-session questionnaire and post session questionnaires were a comparison between lectures and flipped classroom. The post session questionnaire session also consisted of 4 additional questions exclusively on the flipped classroom. All the responses were kept anonymous.

Statistical analysis

The statistical analysis was conducted using SPSS version 30. Descriptive statistics were analysed using the median and range for the likert scale responses. Mean and standard error was used to compare between the cohorts for the multiple choice questions and the short answer question. A Mann-Whitney U test was employed to compare between the two cohorts and within a cohort to compare between lecture and flipped classroom. The p value of < 0.05 was considered to be statistically significant.

Results

(A) Survey

A total of 93 responses (2019–20) and 76 responses (2020–21) were considered for the study. The responses of the survey and academic scores were expressed as median and range. The first 7 questions were comparisons between lecture and FC. For the cohort of 2019–20, and the difference between means was statistically significant for 5 questions ($p < 0.05$) (**Table 1**). Within questions on FC alone, "I recommend flipped classroom for the same course or other courses" showed the highest score for the cohort of 2019–20 (**Table 2**). For the cohort of 2020–21, the difference between the cohorts was statistically significant for 3 questions (**Table 3**).

(B) Academic scores

Comparison was made between academic scores of 3 consecutive years on the same topic taken as

Table 1. Comparison of medians between survey responses on lecture and flipped classroom by Mann-Whitney U test. Responses were recorded on a 5 point Likert scale (1 = strongly disagree to 5 = strongly agree).

Cohort of 2019–20 (n = 93)	Lecture		Flipped classroom		Mann-Whitney U test
Comparison between survey responses on lecture and flipped classroom	Median	Range	Median	Range	p
Lectures help me understand the subject better / Flipped classroom helps me to understand the subject better*	2	4	1	4	<0.001
Lectures help me to remember important concepts/ Flipped classroom helped me to remember important concepts*	2	3	1	4	<0.001
Lectures are interesting and engaging / Flipped Classroom Is Interesting And Engaging*	3	4	1	4	<0.001
During lectures I can discuss my difficulties with my teacher and classmates / During flipped classroom I can discuss my difficulties with my teacher and classmates*	3	3	2	4	<0.001
I feel confident that I will perform better in clinics because of the lecture / I feel confident that I will perform better in clinics because of the flipped classroom	2	3	1	2	<0.001
I feel confident that I will perform better in the theory exam because of the lecture / I feel confident that I will score good marks in the theory exams because of the flipped classroom*	2	3	2	3	0.398
If lectures are uploaded on moodle before coming to the class, I will read and come to the session / I watched all the videos of lectures on moodle before coming to the class*	2	3	3	4	<0.001

* p value < 0.05 was considered to be statistically significant.

Table 2. Median and range of survey responses on flipped classroom(conventional and online) exclusively between the two cohorts. Responses were recorded on a 5 point Likert scale (1 = strongly disagree to 5 = strongly agree).

Survey response on flipped classroom (exclusively)	Cohort 2019–20		Cohort 2020–21		Mann-Whitney U test
	Median	Range	Median	Range	p
I prefer traditional lecture as compared to flipped classroom	2	4	4	4	<0.001
I feel videos of lectures are very useful since they help me to watch them anytime	4	4	4	3	0.612
I recommend flipped classroom for the same course or other courses as well	1	4	4	4	<0.001
I had to put more effort for the flipped classroom as compared to lecture	3	4	4	4	<0.001

Table 3. Comparison of medians between survey responses on online lecture and online flipped classroom by Mann-Whitney U test. Responses were recorded on a 5 point Likert scale (1 = strongly disagree to 5 = strongly agree).

Cohort of 2020–21 (n = 76)	Online lectures		Flipped classroom (online)		Mann-Whitney U test
Comparison between survey responses on lecture and flipped classroom	Median	Range	Median	Range	p
Online Lectures help me understand the subject better / Flipped classroom helps me to understand the subject better*	4	4	5	4	0.001
Lectures help me to remember important concepts / Flipped classroom helped me to remember important concepts*	4	4	5	3	<0.001
Lectures are interesting and engaging / Flipped Classroom Is Interesting And Engaging*	4	4	4	3	<0.001
During lectures I can discuss my difficulties with my teacher and classmates / During flipped classroom I can discuss my difficulties with my teacher and classmates	4	4	4	4	0.591
I feel confident that I will perform better in clinics because of the lecture / I feel confident that I will perform better in clinics because of the flipped classroom	4	4	4	4	<0.001
I feel confident that I will perform better in the theory exam because of the lecture / I feel confident that I will score good marks in the theory exams because of the flipped classroom	4	3	4	4	0.044
If lectures are uploaded on moodle before coming to the class, I will read and come to the session / I watched all the videos of lectures on moodle before coming to the class.	5	2	4	3	0.012

Table 4. (A) Comparison between mean scores and standard error between scores (10 MCQ's and 1 SAQ) for years 2016–17, 2017–18,2018–19 from traditional lectures and (10 MCQ's and 1 SAQ) for 2019–20, 2020–21 of traditional FC and online FC respectively.

Cohort	Mean	Standard error	p
2016–17	65.93	3.56863	0.941
2017–18	73.42	2.68143	
2018–19	70.30	2.87547	
2019–20#	70.11	3.08010	
2020–21	74.12	2.44013	

* p value < 0.05 was considered to be statistically significant.
# MCQ's in the years 2019–20 that were part of the exam conducted online with students at home were not included.

Table 5. Statistics of students access to video's and PPT uploaded prior to FC and lectures.

	Resource	1st phase	2nd phase	3rd phase	Overall hits	Hits Per student	Percentage of students utilizing the resources
FC (F2F)	VRL	164	10	4	178	3.24	71.6%
	PPT	27	26	71	124		
	Total	191	36	75	302		
	p value	0.001					
TL	VRL	0	0	39	39	1.98	58.3%
	PPT	48	20	77	145		
	Total	48	20	116	184		
	p value	0.001					
FC (O)	VRL	99	2	4	105	3.57	74%
	PPT	106	11	53	170		
	Total	205	13	57	275		
	p value	0.001					
OL	VRL	0	0	53	53	2.42	59.7%
	PPT	43	29	62	134		
	Total	43	29	115	187		
	p value	0.001					

VRL – video recorded lectures; PPT – power point presentations; FC(F2F) – flipped classroom with face to face session; TL – traditional onsite lecture; FC(O) – flipped classroom with F2F session conducted online; OL – online lecture.
1st Phase = 20 days from the activity (lecture of FC). 3rd Phase = 20 days prior to the summative period. 2nd phase: Days in between 1st and 3rd phase.

a traditional lecture and scores of 2 years wherein the topic was conducted as FC. The academic scores between all the years showed no statistically significant difference (**Table 4**).

(C) Utilization of resources

The time period during which both FCb (F2F) and FC (O) was implemented, showed significantly increased number of hitsb ($p < 0.001$) compared to the period where summative assessment was conducted or neither of these activities were being conducted (**Table 5**). The hits were highest for the VRL during FC, while the PPT presentations were used the most, closer to the summative assessment ($p < 0.001$) (**Figure 2**).

Discussion

Student perceptions

In the current study, the students' response was overwhelmingly positive on the flipped classroom. Students of both cohorts found the FC interesting and engaging compared to the lecture irrespective of whether it was conducted onsite (F2F) or online. The constructs exclusively on students' perceptions on FC also reported a significant positive opinion on the utility of videos of lectures and a desire to see increased number of sessions of FC in the future.

The questionnaire also included questions designed to evaluate students' perception on edu-

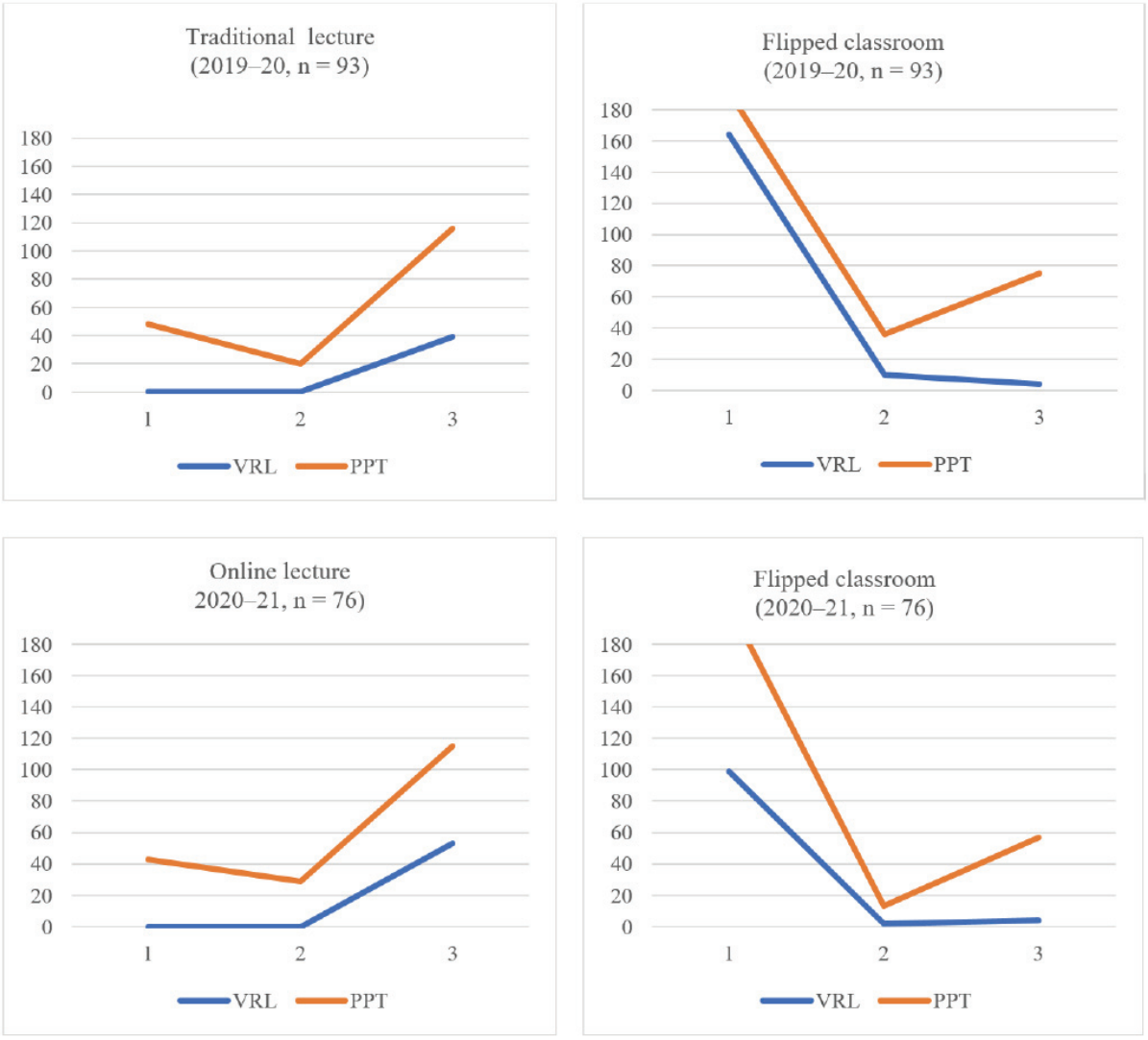

Figure 2. Statistics of students access to video's and PPT uploaded prior to FC and lectures.

cational gains that may be achieved by the flipped classroom. Students believed that FC helps them understand and remember concepts better than lectures. Thus, on both the sub-categories of reaction [13] including instruction method and educational gains, students reacted positively. Systematic reviews [6] and meta-analyses [7,14] across disciplines support the findings from our study that FC is perceived positively by students.

Typically, the F2F is an on-campus session but the COVID-19 pandemic forced these sessions to be online. Online teaching is complex with adaptations required from both: teacher and student. In the current study, faculty were oriented on adapting to online teaching by the medical education unit. The synchronous activities could be conducted online with Zoom. The quiz was conducted on Google Docs and results were displayed and discussed in real time. Breakout rooms were used for the case-based learning sessions to substitute the small group learning F2F sessions effectively.

The online sessions differed from the F2F session in two additional aspects: students had to prepare a concept map for the scenario and the session was allocated marks (2.5% of total marks for didactic component). Concept maps required students to apply their problem-solving skills to address the various possibilities arising from the case scenario.

Self-assessment activities (quiz) promote student engagement [7] and further enhance

the positive perceptions. Small group activities [15], introducing case scenarios [16] and concept maps [17] are both: individually and combined, part of the active learning activities known to enhance learning. The assumption that active learning strategies, 18 conducted in an appropriate way, are in major part responsible for the positive effects of flipped classroom [19], is reinforced by the enhanced student feedback for online FC as well. Nevertheless, apart from the active learning, other aspects such as the faculty orientation, student orientation, resource preparation, focused feedback may in varying degrees have contributed to the student's positive perception.

Unsurprisingly, a small number of students still preferred the lecture (either traditional or online). Moreover, students' scores were high on the opinion that they had to put more effort for the flipped classroom. A lecture mostly involves passive listening and flipped classroom requires students to take responsibility for their learning and hence requires a change in study practices. The reluctance may then be attributed either to resistance to change or a perceived need for instructor to explain the content. Nevertheless, this initial resistance has been reported to ease out over a period of time [20]. Thus, studies with evaluation over long term and larger scale of participation (entire courses and multiple courses in a program) are needed to evaluate if this subgroup can be better engaged by the flipped classroom.

Academic scores

The effect of FC on academic scores was evaluated by comparing historic scores on the selected topic (conducted as a lecture) with FC. The results from our study show that though the average scores were higher for FC, there is no significant difference in the scores between the traditional lecture and FC. The literature on effect of FC on academic scores has been mixed [12] with some reporting a significant increase with introduction of FC [21] while others reported no significant difference [22]. In the current study, the course had both didactic and clinical component, but the FC was implemented for the didactic part alone. It has been argued that the gains in academic scores may not be the appropriate means of measuring gains of FC [23] and the benefits are more measurable in terms of "gains in engagement with academic content, educators and peers, leading to strengthening of lifelong learning" [24].

Study practices

Students were asked prior to a lecture their willingness to read the uploaded resources before reporting to the session. Regardless of the expressed promise of utilizing the online resources prior to lecture, the percentage of students actually utilizing the resources was less than satisfactory. The study habits were further studied by accessing logs of activity on the LMS, Moodle.

During the period associated with FC, a sizeable number of students utilized the online videos with negligible use of PPT's. In the next phase, with no assessments, both video's and PPT usage fell drastically. In the consequent month, with the advent of a summative assessment, the online resource utilization spiked significantly, with a preference for PPT over videos.

Some of the inferences that could be drawn from the analysis are: (a) students' usage of online resources prior to a summative assessment, indicates a tendency to study only with the advent of an assessment(so called binge studying) [25]. Nevertheless, the introduction of FC coincided with increased student engagement with resources uploaded online, irrespective of FC (F2F) or FC online, as compared to the lectures. Even after accounting for the student motivation in FC online due to inclusion of scores in overall grades, the gains in student study practices by weaning them away from "binge studying" seems significant. (b) The introduction of videos/PPT for flipped classroom evinced a keen interest in student. Nevertheless, not more than 75% of the students used the resources. Hence the results of the quiz or assignment are even more important to gauge whether students are well prepared, with the knowledge component (c) Interestingly, the use of PPT was much higher immediately before the exam. This may be explained probably due to the convenience of converting these presentations into handouts and actually writing down notes.

Summary

The summary has been outlined based on Kirkpatrick's scale [26,27], measurable parameter and performance of FC.

Kirkpatrick's scale	Measurable parameter	Performance of FC
Reaction	Student's perception	Positive
Learning	Academic scores	No significant change from lecture
Behavioral change	Student study practices	**Needs more research:** Initial results showed increased utilization of resources by students associated with FC (a practice not noticed with lectures)

Conclusion

Successful student engagement is possible to be achieved even if the F2F session is converted to an online one, by engaging in meaningful active learning. The academic scores with Flipped classroom, online or with F2F, are at least equal to the ones achieved with a lecture. Student behavior of studying only with the advent of a summative assessment can be changed by introduction of FC. The asynchronous activities can be monitored on the LMS, and assurance of learning can be achieved by introduction of self-assessment with or without grading.

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Conflict of interest statement
The authors declare no conflict of interest.

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References

1. Ramnanan CJ, Pound LD. Advances in medical education and practice: student perceptions of the flipped classroom. Advances in medical education and practice. 2017 Jan 13:63-73. doi: 10.2147/AMEP.S109037.
2. Aburahma MH. Do not lose your students in large lectures: A five-step paper-based model to foster students' participation. Pharmacy. 2015 Jul 27;3(3):89-100. doi: 10.3390/pharmacy3030089.
3. Horn MB. The transformational potential of flipped classrooms. Education Next. 2013 Jul 1;13(3):78-9.
4. Kim W. Towards a definition and methodology for blended learning. In The proceedings of workshop on blended learning 2007 Aug 15 (pp. 1-8).
5. Abeysekera L, Dawson P. Motivation and cognitive load in the flipped classroom: definition, rationale and a call for research. Higher education research & development. 2015 Jan 2;34(1):1-4. doi: 10.1080/07294360.2014.934336.
6. Chen F, Lui AM, Martinelli SM. A systematic review of the effectiveness of flipped classrooms in medical education. Medical education. 2017 Jun;51(6):585-97. doi: 10.1111/medu.13272.
7. Hew KF, Lo CK. Flipped classroom improves student learning in health professions education: a meta-analysis. BMC medical education. 2018 Dec;18:1-2. doi: 10.1186/s12909-018-1144-z.
8. Gianoni-Capenakas S, Lagravere M, Pacheco-Pereira C, Yacyshyn J. Effectiveness and perceptions of flipped learning model in dental education: a systematic review. Journal of dental education. 2019 Aug;83(8):935-45. doi: 10.21815/JDE.019.109.
9. Pavanelli R. The flipped classroom: A mixed methods study of academic performance and student perception in EAP writing context. International Journal of Language and Linguistics. 2018;5(2):16-26. doi: 10.30845/ijll.v5n2a3.
10. Iyer P, Aziz K, Ojcius DM. Impact of COVID-19 on dental education in the United States. Journal of dental education. 2020 Jun;84(6):718-22. doi: 10.1002/jdd.12163.
11. Deery C. The COVID-19 pandemic: implications for dental education. Evidence-based dentistry. 2020 Jun;21(2):46-7. doi: 10.1038/s41432-020-0089-3.
12. Vanka A, Vanka S, Wali O. Flipped classroom in dental education: A scoping review. Eur J Dent Educ. 2020 May 1;24(2):213-26. doi: 10.1111/eje.12487.
13. Alliger GM, Tannenbaum SI, Bennett Jr W, Traver H, Shotland A. A meta-analysis of the relations among training criteria. Personnel psychology. 1997 Jun;50(2):341-58. doi: 10.1111/j.1744-6570.1997.tb00911.x.
14. Betihavas V, Bridgman H, Kornhaber R, Cross M. The evidence for 'flipping out': a systematic review of the flipped classroom in nursing education. Nurse Educ Today. 2016;38:15-21. doi: 10.1016/j.nedt.2015.12.010.
15. Johnson DW. Cooperative learning methods: A meta-analysis. 2000.
16. Varthis S, Anderson OR. Students' perceptions of a blended learning experience in dental education. European Journal of Dental Education. 2018 Feb;22(1):e35-41. doi: 10.1111/eje.12253.
17. Darmofal DL, Soderholm DH, Brodeur DR. Using concept maps and concept questions to enhance conceptual understanding. In32nd annual frontiers in education 2002 Nov 6 (Vol. 1, pp. T3A-T3A). IEEE. doi: 10.1109/FIE.2002.1157954.
18. Prince M. Does active learning work? A review of the research. J Eng Educ Res. 2004;93:223-232. doi: 10.1002/j.2168-9830.2004.tb00809.x.
19. Jensen JL, Kummer TA, Godoy PD. Improvements from a flipped classroom may simply be the fruits of

active learning. CBE—Life Sciences Education. 2015 Mar 2;14(1):ar5. doi: 10.1187/cbe.14-08-0129.
20. Bohaty BS, Redford GJ, Gadbury-Amyot CC. Flipping the classroom: assessment of strategies to promote student-centered, self-directed learning in a dental school course in pediatric dentistry. J Dent Educ. 2016;80(11):1319-1327. doi: 10.1002/j.0022-0337.2016.80.11.tb06217.x.
21. Deslauriers L, Schelew E, Wieman C. Improved learning in a large-enrollment physics class. Science. 2011 May 13;332(6031):862-4. doi: 10.1126/science.1201783.
22. Gillette C, Rudolph M, Kimble C, Rockich-Winston N, Smith L, Broedel-Zaugg K. A meta-analysis of outcomes comparing flipped classroom and lecture. American journal of pharmaceutical education. 2018 Jun 1;82(5):6898. doi: 10.5688/ajpe6898.
23. McLean S, Attardi SM, Faden L, Goldszmidt M. Flipped classrooms and student learning: not just surface gains. Advances in physiology education. 2016 Mar;40(1):47-55. doi: 10.1152/advan.00098.2015.
24. Smallhorn M. The flipped classroom: A learning model to increase student engagement not academic achievement. Student Success. 2017 Jul;8(2):43-53. doi: 10.5204/ssj.v8i2.381.
25. Gadbury-Amyot CC, Redford GJ, Bohaty BS. Dental students' study habits in flipped/blended classrooms and their association with active learning practices. J Dent Educ. 2017;81(12):1430-1435. doi: 10.21815/JDE.017.103.
26. Kirkpatrick D, Kirkpatrick J. Evaluating training programs: The four levels. Berrett-Koehler Publishers; 2006.
27. Praslova L. Adaptation of Kirkpatrick's four level model of training criteria to assessment of learning outcomes and program evaluation in higher education. Educational assessment, evaluation and accountability. 2010 Aug;22:215-25. doi: 10.1007/s11092-010-9098-7.

ORIGINAL PAPER

JMS Journal of Medical Science

Body composition analysis in patients with Hashimoto's disease and vitamin D deficiency

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ABSTRACT

Introduction. Hashimoto thyroiditis (HT) is one of the most common organ-specific autoimmune diseases. This autoimmune response disrupts thyroid function, affecting biochemical processes and metabolism, with symptoms often including weight gain and easy fatigue. Vitamin D has so far been considered only a key regulator of calcium-phosphate metabolism. However, it is now considered a pleiotropic substance and increasingly published data indicate that it also plays a role in immune modulation and metabolic health.
Aim. The study aimed to assess anthropometric measures, body composition, and muscle strength in patients with HT, correlate these parameters with serum 25(OH)D levels, and evaluate the impact of vitamin D supplementation.
Material and methods. The study included 80 female patients, aged ≥18 years, divided into an HT group (n = 51) and a Control Group with non-toxic diffuse or nodular goiter (n = 29). Vitamin D supplementation was administered based on the initial 25(OH)D concentration, at a dose of 6000 IU or 4000 IU daily for 3 months to patients with 25(OH)D concentration < 20 ng/ml and ≥ 20 ng/ml, respectively. Anthropometric and body composition measurements, as well as blood tests for 25(OH)D, TSH were performed at baseline and after supplementation.
Results. Both groups showed significant increases in 25(OH)D levels post-supplementation. In the HT group, lean body mass and muscle strength improved significantly (p = 0.002 and p = 0.02, respectively). In the Control Group, muscle strength increased (p = 0.01) and hip circumference decreased slightly (p = 0.03). No significant differences were found in body composition between the groups, although women with HT had larger hip circumferences. Correlation analysis revealed a moderately strong inverse relationship between baseline 25(OH)D levels and BMI in the Control Group (R = -0.44; p = 0.04), with no other significant associations identified.
Conclusion. Vitamin D supplementation effectively increased serum 25(OH)D levels and improved muscle strength and lean body mass in women with HT. Further research is needed to explore the mechanisms by which vitamin D may impact metabolic and immune health in HT patients.

Introduction

Hashimoto thyroiditis (HT), also known as chronic autoimmune thyroiditis, along with Graves' disease, is one of the most common organ-specific autoimmune diseases. HT is associated with the infiltration of the thyroid gland by T lymphocytes and B lymphocytes. The latter cells produce characteristic antibodies against thyroperoxidase (Anti-TPO) and thyroglobulin (Anti-TG), ultimately leading to thyroid dysfunction. The prevalence of the disease increases with age and women have over ten times higher risk of developing it [1].

Thyroid hormones influence many biochemical processes in the body, participating in thermogenesis, controlling the rate of basal metabolism, and regulating the metabolism of carbohydrates, proteins, and fats. Therefore, patients with HT often experience symptoms affecting various organs and systems, including excessive weight gain, loss of energy, decreased physical function, depending on the degree of thyroid dysfunction [2].

Vitamin D performs various essential functions in the body. One of its main tasks is the regulation of calcium-phosphate metabolism. However, its role extends beyond the skeletal system. In the form of calcitriol, a steroid-like hormone, it influences the immune system, muscle function, and may also affect metabolic processes, such as weight regulation [3]. Vitamin D deficiency has been linked to numerous chronic diseases, including obesity, metabolic syndrome, hypertension and type 2 diabetes [4]. Many scientific reports postulate that adipose tissue is a source of pro-inflammatory cytokines, including interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α) and stimulation of Th17 lymphocytes involved in the initiation of autoimmune diseases [5]. In opposition to this process is Vitamin D, which contributes to changing the immune state from pro-inflammatory to immunological tolerance. The effect of vitamin D on CD4+ T cells is mainly to inhibit the proliferation of the Th1 line and the cytokines they produce. The response of Th2 line lymphocytes is enhanced by promoting the production of anti-inflammatory cytokines: IL-5, IL-10, TGF-β [6]. Additionally, inhibition of the expression of IL-6, the main factor stimulating Th17 lymphocytes involved in autoimmune reactions was observed [7]. This has a potential beneficial effect in people with abnormal body composition and excess fat tissue.

Aim

The aim of the present study was to analyze selected anthropometric measures and body composition in patients with HT, correlate these parameters with serum 25OHD concentration, and assess the impact of vitamin D supplementation on the aforementioned parameters.

Material and methods

Eighty patients under the care of the Endocrinology Outpatient Clinic at the Regional Center for Menopause and Osteoporosis of the The Military Medical Academy Memorial Teaching Hospital in Lodz – Central Veterans Hospital, aged ≥ 18 years, were enrolled in the study. There were 51 patients diagnosed with Hashimoto's disease (Study Group) and 29 patients with non-toxic diffuse goiter or nodular goiter (Control Group). The patients had not previously supplemented with vitamin D or had a break in supplementation of at least 6 months. The study was conducted from September to May.

Patients were diagnosed based on clinical symptoms and biochemical test results. The diagnosis of Hashimoto's disease was established based on anti-thyroid peroxidase (Anti-TPO) and/or anti-thyroglobulin (Anti-TG) antibody concentrations above the upper limit of reference range (positive result), a typical thyroid ultrasound image, and the exclusion of Graves' disease. The Study Group (with Hashimoto's disease) included patients in a euthyroid clinical state undergoing levothyroxine replacement therapy if necessary. The Control Group (without Hashimoto's disease) comprised patients with diagnosed non-toxic diffuse or nodular goiter with low (negative) concentrations of anti-thyroid antibodies.

Patients with chronic conditions affecting vitamin D absorption and metabolism, such as malabsorption syndrome, chronic kidney disease, liver diseases, hyperparathyroidism, Cushing's syndrome/disease, hypogonadism, hyperprolactinemia, other autoimmune diseases, active

cancer, and/or undergoing chemotherapy, as well as those taking medications affecting vitamin D metabolism were excluded from the study.

After qualification and obtaining consent to participate, basic anthropometric measurements (weight, height, waist and hip circumference) were performed. Body composition analysis was conducted using the Bodystat®1500 device. Blood samples were collected for the determination of Total Vitamin D [25(OH)D total], TSH, anti-TPO, and anti-TG levels.

Following the initial procedures, patients were categorized into two groups based on their serum 25(OH)D levels:

- Group A: Patients with vitamin D levels ≥ 20 ng/mL,
- Group B: Patients with vitamin D levels < 20 ng/mL.

Depending on the initial serum 25(OH)D concentration, patients received oral vitamin D (cholecalciferol) once daily for 3 months, at a dose of 4000 IU/ daily for group A and 6000 IU/ daily for group B.

After 3 months of vitamin D supplementation, anthropometric measurements, body composition analysis and laboratory tests were repeated.

Anthropometric measurements and body composition analysis

Anthropometric measurements were conducted with patients fasting and wearing light clothing. Weight and height were measured with an accuracy of 0.1 kg and 0.1 cm, respectively, and BMI was calculated as weight (kg)/height (m^2). Obesity was defined as BMI ≥ 30 kg/m^2. Waist circumference was measured halfway between the lowest rib and the iliac crest, and hip circumference was measured at the level of the pubic bone, with the criterion for obesity being a waist circumference ≥ 80 cm and WHR < 0.8 indicating gluteofemoral obesity (pear-shaped), while a waist circumference ≥ 80 cm and WHR ≥ 0.8 indicated abdominal obesity (apple-shaped).

Body composition was assessed using bioimpedance measurements with the Bio-impedance Analyser (Bodystat® 1500). Measurements included: fat mass (kg), percentage fat (%Fat), lean mass (kg), and percentage body water (%Water). Muscle strength measurements were conducted using a spring dynamometer for the dominant hand with results expressed in kilograms.

Laboratory tests

Vitamin D concentration was determined using an electrochemiluminescence test, while TSH concentration was determined using third-generation immunometric tests. Anti-TPO and anti-TG antibody concentrations were measured using an enhanced chemiluminescence method.

Statistical analysis

Statistical analysis was performed using R-4.3.0 software. Initial parameter concentrations were compared using the Student's t-test or the non-parametric Mann-Whitney test. The Shapiro-Wilk test was employed to assess the normality of parameter distributions. The significance threshold was $p = 0.05$. To examine correlations Spearman and Pearson correlation coefficients and a test for the significance of correlation were used. Changes in evaluated parameters between two time points (baseline and 3 months) were analyzed using the paired Student's t-test or the Wilcoxon non-parametric test with a significance threshold of $p = 0.05$.

Results

The study included 80 women: 29 in the Control Group (women without Hashimoto's disease) aged 33–73 years and 51 in the Study Group (women with Hashimoto's disease) aged 22–78 years. All women were administered vitamin D (Vitrum D3 forte; Takeda) based on their 25(OH)D initial concentration with doses of 4000 or 6000 IU daily. In the Study Group, 32 women were postmenopausal (63%), while in the Control Group, 17 women were postmenopausal (59%). In the group of patients with HT (Study Group), suboptimal 25(OH)D levels were observed in 50%, while in patients without HT (Control Group), 25(OH)D levels < 20 ng/ml were observed in 49% of participants. No statistically significant difference was found between the groups in this regard. In the conducted study, the mean 25(OH)D concentration in both groups was similar, amounting to 19.97 ng/ml and 21.36 ng/ml, respectively.

Women without HT had a significantly smaller hip circumference compared to patients with HT ($p = 0.049$), but the average BMI in both groups

was similar and within the overweight range (28 kg/m^2 vs. 27 kg/m^2). In the Hashimoto's disease group, 41% of women were obese, whereas in the group without Hashimoto's disease, the percentage of women with BMI ≥ 30 kg/m^2 was lower, constituting 27% (**Figure 1**). WHR values

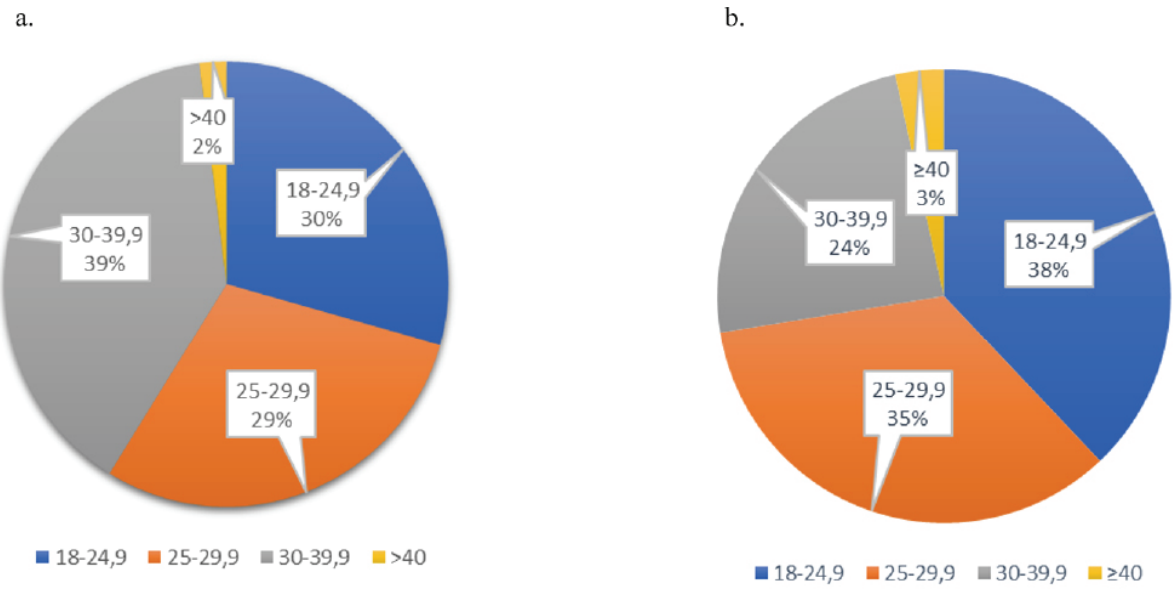


Figure 1. Distribution of BMI (kg/m^2) in the Study Group (a) and Control Group (b).

Table 1. Comparative Characteristics of the Study Group and Control Group at Baseline.

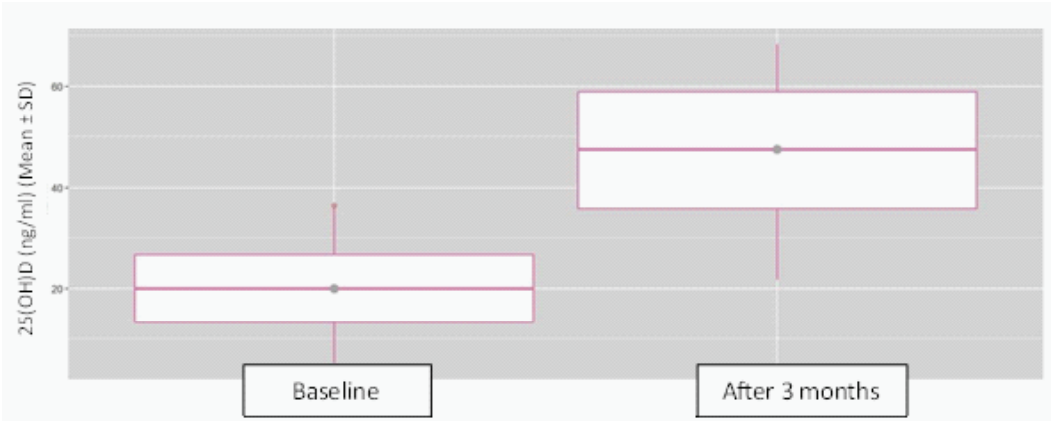
	STUDY GROUP			CONTROL GROUP			
Parameter	**Mean**	**SD**	**Std.error**	**Mean**	**SD**	**Std.error**	**P**
Age	53.98	15.04	29.11	54.35	13.58	2.52	0.93
Weight (kg)	76.40	14.97	2.10	73.13	13.51	2.51	0.32
Height (m)	1.63	0.06	0.01	1.63	0.04	0.01	0.55
BMI (kg/m^2)	28.37	5.44	0.76	27.90	4.92	0.93	0.70
Waist (cm)	87.08	15.51	2.17	81.50	12.30	2.33	0.08
Hips (cm)	106.76	11.75	1.65	102.71	12.47	2.36	**0.049**
WHR	0.81	0.07	0.01	0.79	0.06	0.01	0.20
25(OH)D (ng/ml)	19.97	6.72	0.94	21.36	10.90	2.02	0.54
TSH (U/l)	2.01	1.19	0.17	1.19	0.62	0.12	**0.002**
anti-TPO (IU/ml)	297.98	214.40	30.02	15.83	9.19	1.71	**0.001**
anti-TG (IU/ml)	762.85	1098.48	153.82	31.13	39.18	7.28	**0.001**
Muscle strenght (kg)	37.80	6.08	0.86	36.97	5.88	1.11	0.93
Fat mass (kg)	30.47	11.93	1.67	27.15	10.75	2.03	0.21
Fat mass (%)	38.37	9.12	1.28	36.34	1.54	8.17	0.32
Lean mass (kg)	46.04	5.60	0.79	45.29	5.57	1.05	0.36
Body water (%)	47.34	5.91	0.83	49.40	5.48	1.04	0.13

were comparable between both groups. No differences were observed in terms of body composition and muscle strength between the Study Group and the Control Group. Patient characteristics at baseline are presented in **Table 1**.

After 3 months of vitamin D supplementation, serum 25(OH)D concentrations significantly increased in all women, indicating good compliance (**Figure 2**). In the group of patients with HT, the average concentration was 47.460 ± 1.61 SD ng/dl, while in the group of patients without HT, it was 49.085 ± 2.51 SD ng/dl.

In patients with HT, a statistically significant increase in lean body mass (p = 0.002) and

a.



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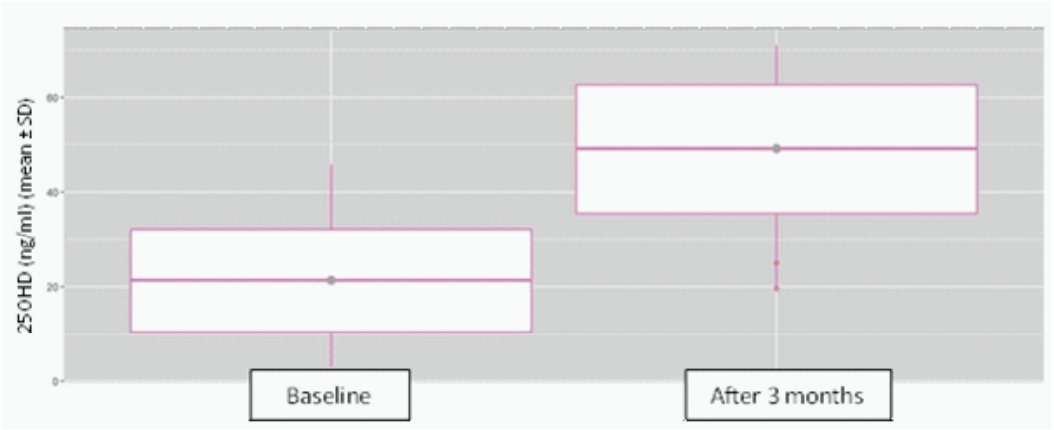

Figure 2. Change in 25(OH)D concentration after 3 months in the Study Group (p = 0.001) (a) and in the Control Group (p = 0.001) (b).

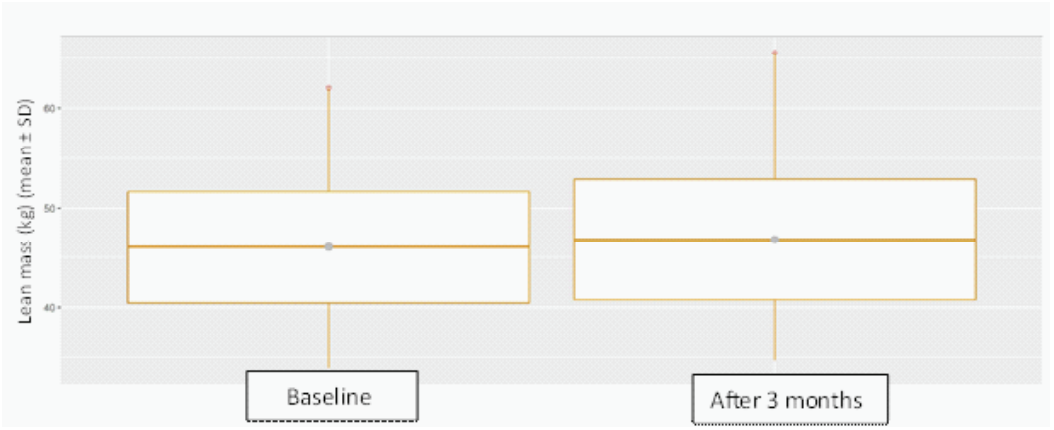

Figure 3. Change in Lean Body Mass (kg) after 3 months in the Study Group (p = 0.002).

muscle strength (p = 0.02) was observed (**Figures 3** and **4**). On the other hand, women without HT showed improvements in muscle strength (p = 0.01) and a reduction in mean hip circumference by 0.5 cm (p = 0.03) (**Figures 4** and **5**), but no impact on parameters related to lean body mass was noted.

Additionally, the correlation analysis between baseline 25(OH)D concentration and anthropometric parameters (waist circumference, hip circumference, BMI, WHR) and body composition parameters did not reveal statistically significant relationships. Only a moderately strong, inverse relationship between 25(OH)D concentration and

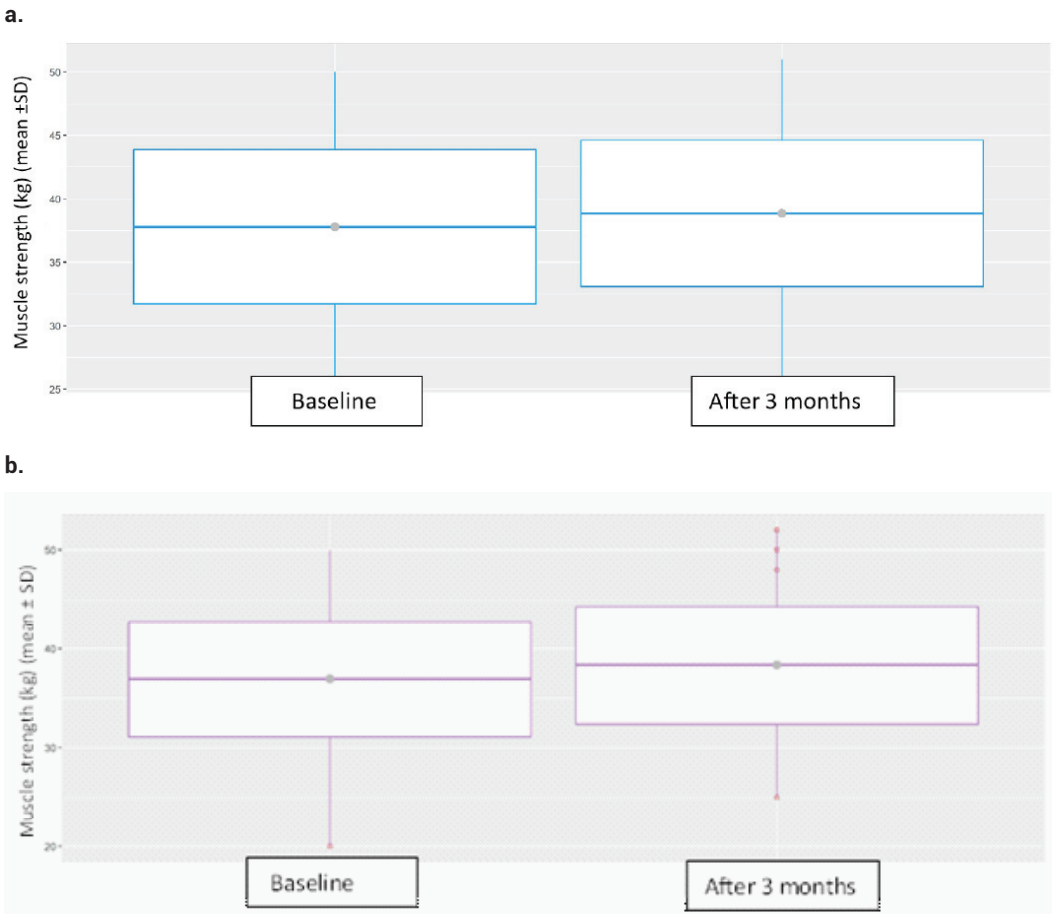

Figure 4. Change in muscle strength (kg) after 3 months in the Study Group (p = 0.02) (a) and in the Control Group (p = 0.01) (b).

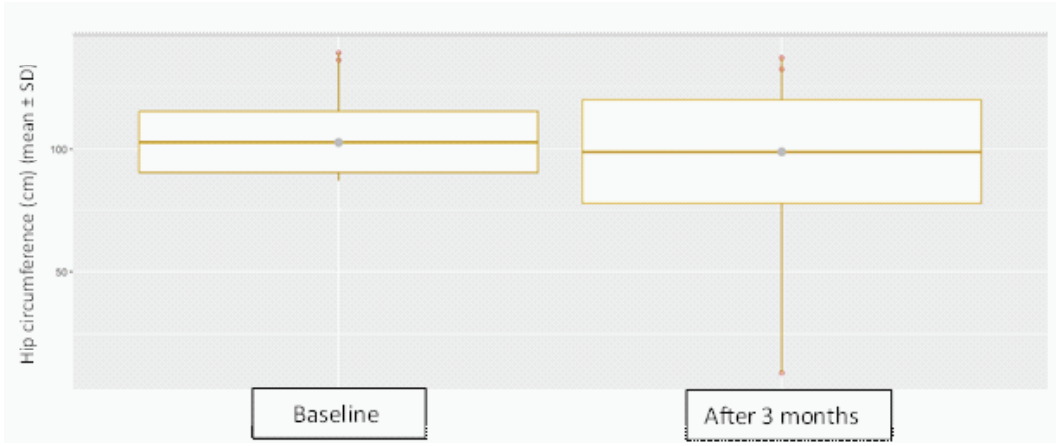

Figure 5. Change in hip circumference (cm) after 3 months in the Control Group (p = 0.03).

BMI in the Control Group was observed (R = -0.44; p = 0.04) (data not shown).

Correlations between the concentration of 25(OH)D and the tested parameters were also analyzed after 3 months of supplementation, showing no statistically significant results.

A correlation analysis between TSH levels and the assessed anthropometric parameters at the baseline and after 3 months was performed, but no statistically significant relationships were observed (data not shown).

Discussion

Vitamin D deficiency is common in the polish population. In a population study by Płudowski et al., involving 5775 adult volunteers from 22 cities in Poland, it was found that 89.9% of them had a deep deficit or insufficient supply of vitamin D, i.e., 25(OH)D levels below 30 ng/ml [8]. In our study suboptimal 25(OH)D levels were observed in 50% patients with HT, while in patients without HT 25(OH)D levels < 20 ng/ml were observed in 49% of participants.

In recent years, many researchers have analyzed the role of vitamin D in the etiopathogenesis of obesity. Studies have shown lower serum 25-hydroxyvitamin D levels in obese individuals compared to those with normal body weight [9–11]. This is confirmed by a meta-analysis conducted by Saneei et al., which found a statistically significant inverse relationship between BMI and serum 25(OH)D levels [12]. Vimaleswaran et al., using Mendelian randomization, suggested a relationship between BMI and serum 25(OH) D levels. It was shown that an increase in BMI is linearly associated with a decrease in the concentration of 25(OH)D in serum. However, there was no evidence of changes in BMI values with an increase in 25(OH)D levels in serum [13]. The mechanism of vitamin D deficiency is explained, among other factors, by increased accumulation of vitamin D in muscle, adipose tissue, and the liver. In obese patients, the content of 25(OH)D in these tissues increases, resulting in a lower level of vitamin D in the serum [14,15]. Additionally, the stimulation of parathyroid hormone release due to relative vitamin D deficiency leads to calcium influx into adipocytes, stimulating lipogenesis [16]. In our study, the mean 25(OH)D concentration in patients with HT (Study Group) was 19.97 ng/ml, and in patients without HT (Control Group) it was 21.36 ng/ml. We did not find a statistically significant difference between the groups and there was no relationship between BMI and serum 25(OH)D levels in women with HT. However, in women without autoimmune thyroid disease, we observed a moderately strong, negative trend between these parameters. The narrow range of serum vitamin D levels in our patients may have influenced the observation of a possible relationship between 25(OH)D and BMI. Similarly, Baradaran et al. did not confirm such a relationship in a population of 259 healthy adults. The authors concluded that the relationship between BMI and serum 25(OH)D levels should also be considered in correlation with the age of the studied population [17].

Vitamin D deficiency may be also related to an improper body composition, leading to a reduction in lean body mass through the loss of bone mineral density, decreased muscle mass, strength, and function [18]. Randomized, placebo-controlled studies have shown that vitamin D supplementation can slow down the rate of bone loss in older individuals [19]. A study of 341 healthy individuals found that lower vitamin D levels are associated with increased overall body fat mass measured by bioimpedance, regardless of BMI [20]. Karefylakis et al. demonstrated that vitamin D therapy at a dose of 2000 IU per day for 6 months had no effect on final BMI and fat content measured by bioelectrical impedance analysis in 40 overweight and obese patients [21]. The analysis of twenty randomized controlled trials conducted by Karampel et al. concluded that, compared to placebo, vitamin D supplementation did not cause a significant reduction in BMI, WC and WHR. However, in subgroups of women, in studies from the Asian region and with an intervention duration of ≥ 6 months, a favorable and significant reduction of BMI and WC ($p < 0.026$) were observed [22]. Based on scientific evidence and recommendations [23] indicating that the time required to achieve optimal serum 25(OH) D concentrations is 8–12 weeks, the observation period in our study was set to 3 months. Patients received higher doses of vitamin D3 to reach optimal serum levels within this time, then the dosage was reduced to 2000 IU per day. In our population, both in women with HT and in healthy

women, we did not find a relationship between 25(OH)D levels and the amount of body fat. Additionally, cholecalciferol supplementation, similar to the study mentioned above, did not result in significant changes in this regard. This is also confirmed by a meta-analysis conducted by Golzarand et al., which showed that 25(OH)D levels were inversely correlated with the percentage of body fat, but cholecalciferol supplementation had no effect on its reduction over time [24].

Sun et al. investigated the effect of cholecalciferol supplementation on lean body mass in a population of 45 healthy individuals who received 420 IU of vitamin D3 daily, while 47 received a placebo. After a one-year observation period, an increase in lean body mass was observed in the intervention group, but no effect of treatment on other body composition indices was observed [25]. In a population of similar to our group 80 postmenopausal Brazilian women with hypertension, vitamin D supplementation (1000 IU) was observed to be a significant protective factor against the development of sarcopenia, causing a significant increase in muscle strength and counteracting the progressive loss of lean body mass. The intervention period was 9 months, and lean body mass was measured using dual-energy X-ray absorptiometry (DXA) [26]. Similar conclusions were reached by a meta-analysis of 13 randomized, placebo-controlled trials, which showed that vitamin D supplementation increases muscle strength in postmenopausal women [27]. In contrast, Manson et al., in a study involving 25,871 healthy individuals, found no effect of vitamin D supplementation (2000 IU) on body weight, BMI and fat-free mass. The observed effects were independent of gender, race or baseline 25(OH)D concentration [28]. In our study, we demonstrated that in patients with HT, there was a statistically significant increase in lean body mass ($p = 0.002$) and muscle strength ($p = 0.02$), while in healthy women, only improvements in muscle strength were observed ($p = 0.01$). This may play a significant role, particularly in obese patients and those over the age of 75, for whom the recommended prophylactic dose of vitamin D should be higher than in the general population.

In order to eliminate the influence of decompensated hypothyroidism on the studied parameters, all women of study our population with HT remained euthyroid, and both groups showed no differences in the studied anthropometric parameters at the baseline. Wolf et al. found that in patients after thyroidectomy with short-term uncompensated hypothyroidism, the body water content was significantly lower, while the fat tissue percentage was significantly higher compared to a group without these disorders [29]. After the initiation of levothyroxine therapy, body composition did not significantly differ between the study and control groups. In the study by Mousa et al., comparing 99 patients with Hashimoto's thyroiditis (HT) and normal TSH values with 202 healthy women and men, no difference in BMI or fat content measured by bioimpedance was observed [30]. On the other hand, Adamska et al. demonstrated that women with HT had a higher percentage of body fat, measured by bioimpedance, but did not exhibit greater android, gynoid fat, or visceral fat mass compared to the control group, However, the average age of individuals in this study was much lower than in our population [31].

Numerous studies have explored the potential impact of slight changes in thyroid function in euthyroid patients on anthropometric parameters and related indices [32–39]. Findings regarding the relationship between serum Thyroid-Stimulating Hormone (TSH) within reference values and Body Mass Index (BMI) remain conflicting. Some publications confirm a positive correlation between serum TSH levels and BMI [34,35], while others do not observe such correlations [36–38]. In our study, we also did not find a relationship between TSH concentration and BMI, as well as fat and lean body mass. Analyzing the influence of BMI on the hormonal status of patients, it has been shown that obese individuals have higher TSH levels [39]. This is likely due to increased release of adipokines and proinflammatory cytokines from adipose tissue, promoting the recruitment of additional immune cells and exacerbating systemic inflammation [40–42]. The rise in TSH levels in obese individuals is also associated with the influence of leptin, adiponectin, and resistin on pro-thyrotropin-releasing hormone (pro-TRH) production in the hypothalamus. These adipokines produced by adipose tissue may participate in the interaction between adipocytes and the hypothalamus, resulting in increased release of TRH and TSH and consequently, the production and secretion of free thyroxine (FT4) [42–44].

Our study had some limitations. Blood samples were collected between September and May to avoid interference with sun exposure; therefore, serum 25(OH)D levels did not reflect the full vitamin D status throughout the year. Other limitations include a relatively short observation period, small study population and the use of a less accurate method for assessing body composition, such as bioimpedance measurement, compared to DXA or MRI.

Conclusions

In euthyroid patients with HT, the values of anthropometric parameters and body composition were similar to those in healthy people. Obesity and vitamin D deficiency are common, which is also confirmed in the population of HT patients. Vitamin D supplementation may and even should be recommended to patients with HT due to its potentially beneficial effect on the increase in lean body mass and muscle strength, as well as its anti-inflammatory effect. Further research is needed on the effect of vitamin D on metabolic processes in patients with autoimmune thyroid diseases

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Bioethics Committee approval
The research project was approved by the Bioethics Committee of the Medical University of Lodz (resolution RNN/02/17/KE).

Conflict of interest statement
The authors declare no conflict of interest.

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References

1. Mincer DL, Jialal I. StatPearls: Hashimoto Thyroiditis. Treasure Island (FL); 2024.
2. Kawicka A, Regulska-Ilow B. Metabolic disorders and nutritional status in autoimmune thyroid diseases. Postepy Hig Med Dosw (Online). 2015;69:80–90. doi: 10.5604/17322693.1136383.
3. Mendes MM, Botelho PB, Ribeiro H. Vitamin D and musculoskeletal health: Outstanding aspects to be considered in the light of current evidence. Endocr Connect. 2022 Sep 26;11(10):e210596. doi: 10.1530/EC-21-0596.
4. Wimalawansa SJ. Associations of vitamin D with insulin resistance, obesity, type 2 diabetes, and metabolic syndrome. J Steroid Biochem Mol Biol. 2018;175:177–89. doi: 10.1016/j.jsbmb.2016.09.017.
5. Galușca D, Popoviciu MS, Babeș EE, Vidican M, Zaha AA, Babeș VV, et al. Vitamin D Implications and Effect of Supplementation in Endocrine Disorders: Autoimmune Thyroid Disorders (Hashimoto's Disease and Grave's Disease), Diabetes Mellitus and Obesity. Medicina (Kaunas) 2022. doi: 10.3390/medicina58020194.
6. Athanassiou L, Mavragani CP, Koutsilieris M. The Immunomodulatory Properties of Vitamin D. Mediterr J Rheumatol. 2022;33:7–13. doi: 10.31138/mjr.33.1.7.
7. Corrado A, Rotondo C, Sanpaolo ER, Altomare A, Maruotti N, Cici D, Cantatore FP. 1,25OH-Vitamin D3 and IL-17 Inhibition Modulate Pro-Fibrotic Cytokines Production in Peripheral Blood Mononuclear Cells of Patients with Systemic Sclerosis. Int J Med Sci. 2022;19:867–77. doi: 10.7150/ijms.70984.
8. Płudowski P, Ducki C, Konstantynowicz J, Jaworski M. Vitamin D status in Poland. Pol Arch Med Wewn. 2016;126:530–9. doi: 10.20452/pamw.3479.
9. Ding C, Parameswaran V, Blizzard L, Burgess J, Jones G. Not a simple fat-soluble vitamin: Changes in serum 25-(OH)D levels are predicted by adiposity and adipocytokines in older adults. J Intern Med. 2010;268:501–10. doi: 10.1111/j.1365-2796.2010.02267.x.
10. Vashi PG, Lammersfeld CA, Braun DP, Gupta D. Serum 25-hydroxyvitamin D is inversely associated with body mass index in cancer. Nutr J. 2011;10:51. doi: 10.1186/1475-2891-10-51.
11. Tosunbayraktar G, Bas M, Kut A, Buyukkaragoz AH. Low serum 25(OH)D levels are associated to hıgher BMI and metabolic syndrome parameters in adult subjects in Turkey. Afr Health Sci. 2015;15:1161–9. doi: 10.4314/ahs.v15i4.15.
12. Saneei P, Salehi-Abargouei A, Esmaillzadeh A. Serum 25-hydroxy vitamin D levels in relation to body mass index: A systematic review and meta-analysis. Obes Rev. 2013;14:393–404. doi: 10.1111/obr.12016.
13. Vimaleswaran KS, Berry DJ, Lu C, Tikkanen E, Pilz S, Hiraki LT, et al. Causal relationship between obesity and vitamin D status: Bi-directional Mendelian randomization analysis of multiple cohorts. PLoS Med. 2013;10:e1001383. doi: 10.1371/journal.pmed.1001383.
14. Park CY, Han SN. The Role of Vitamin D in Adipose Tissue Biology: Adipocyte Differentiation, Energy Metabolism, and Inflammation. J Lipid Atheroscler. 2021;10:130–44. doi: 10.12997/jla.2021.10.2.130.
15. Walsh JS, Bowles S, Evans AL. Vitamin D in obesity. Curr Opin Endocrinol Diabetes Obes. 2017;24:389–94. doi: 10.1097/MED.0000000000000371.
16. McCarty MF, Thomas CA. PTH excess may promote weight gain by impeding catecholamine-induced lipolysis-implications for the impact of calcium, vitamin D, and alcohol on body weight. Med Hypotheses. 2003;61:535–42. doi: 10.1016/s0306-9877(03)00227-5.

17. Baradaran A, Behradmanesh S, Nasri H. Association of body mass index and serum vitamin D level in healthy Iranian adolescents. Endokrynol Pol. 2012;63:29–33.
18. Delinocente MLB, Luiz MM, Oliveira DC de, Souza AF de, Ramírez PC, Oliveira Máximo R de, et al. Are Serum 25-Hydroxyvitamin D Deficiency and Insufficiency Risk Factors for the Incidence of Dynapenia? Calcif Tissue Int. 2022;111:571–9. doi: 10.1007/s00223-022-01021-8.
19. Moreira-Pfrimer LDF, Pedrosa MAC, Teixeira L, Lazaretti-Castro M. Treatment of vitamin D deficiency increases lower limb muscle strength in institutionalized older people independently of regular physical activity: A randomized double-blind controlled trial. Ann Nutr Metab. 2009;54:291–300. doi: 10.1159/000235874.
20. Al Hayek S, Matar Bou Mosleh J, Ghadieh R, El Hayek Fares J. Vitamin D status and body composition: A cross-sectional study among employees at a private university in Lebanon. BMC Nutr. 2018;4:31. doi: 10.1186/s40795-018-0239-6.
21. Karefylakis C, Särnblad S, Ariander A, Ehlersson G, Rask E, Rask P. Effect of Vitamin D supplementation on body composition and cardiorespiratory fitness in overweight men-a randomized controlled trial. Endocrine. 2018;61:388–97. doi: 10.1007/s12020-018-1665-6.
22. Karampela I, Sakelliou A, Vallianou N, Christodoulatos G-S, Magkos F, Dalamaga M. Vitamin D and Obesity: Current Evidence and Controversies. Curr Obes Rep. 2021;10:162–80. doi: 10.1007/s13679-021-00433-1.
23. Płudowski P, Kos-Kudła B, Walczak M, Fal A, Zozulińska-Ziółkiewicz D, Sieroszewski P, et al. Guidelines for Preventing and Treating Vitamin D Deficiency: A 2023 Update in Poland. Nutrients 2023. doi: 10.3390/nu15030695.
24. Golzarand M, Hollis BW, Mirmiran P, Wagner CL, Shab-Bidar S. Vitamin D supplementation and body fat mass: A systematic review and meta-analysis. Eur J Clin Nutr. 2018;72:1345–57. doi: 10.1038/s41430-018-0132-z.
25. Sun X, Tanisawa K, Zhang Y, Ito T, Oshima S, Higuchi M, Cao Z-B. Effect of Vitamin D Supplementation on Body Composition and Physical Fitness in Healthy Adults: A Double-Blind, Randomized Controlled Trial. Ann Nutr Metab. 2019;75:231–7. doi: 10.1159/000504873.
26. Cangussu LM, Nahas-Neto J, Orsatti CL, Bueloni-Dias FN, Nahas EAP. Effect of vitamin D supplementation alone on muscle function in postmenopausal women: A randomized, double-blind, placebo-controlled clinical trial. Osteoporos Int. 2015;26:2413–21. doi: 10.1007/s00198-015-3151-9.
27. Zhang J-L, Poon CC-W, Wong M-S, Li W-X, Guo Y-X, Zhang Y. Vitamin D Supplementation Improves Handgrip Strength in Postmenopausal Women: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. Front Endocrinol (Lausanne). 2022;13:863448. doi: 10.3389/fendo.2022.863448.
28. Manson JE, Cook NR, Lee I-M, Christen W, Bassuk SS, Mora S, et al. Vitamin D Supplements and Prevention of Cancer and Cardiovascular Disease. N Engl J Med. 2019;380:33–44. doi: 10.1056/NEJMoa1809944.
29. Wolf M, Weigert A, Kreymann G. Body composition and energy expenditure in thyroidectomized patients during short-term hypothyroidism and thyrotropin-suppressive thyroxine therapy. Eur J Endocrinol. 1996;134:168–73. doi: 10.1530/eje.0.1340168.
30. Mousa U, Bozkuş Y, Kut A, Demir CC, Tutuncu NB. Fat distribution and metabolic profile in subjects with Hashimoto`s thyroiditis. Acta Endocrinol (Buchar). 2018;14:105–12. doi: 10.4183/aeb.2018.105.
31. Adamska A, Popławska-Kita A, Siewko K, Łebkowska A, Krentowska A, Buczyńska A, et al. Body Composition and Serum Anti-Müllerian Hormone Levels in Euthyroid Caucasian Women With Hashimoto Thyroiditis. Front Endocrinol (Lausanne). 2021;12:657752. doi: 10.3389/fendo.2021.657752.
32. Knudsen N, Laurberg P, Rasmussen LB, Bülow I, Perrild H, Ovesen L, Jørgensen T. Small differences in thyroid function may be important for body mass index and the occurrence of obesity in the population. J Clin Endocrinol Metab. 2005;90:4019–24. doi: 10.1210/jc.2004-2225.
33. Dvořáková M, Hill M, Čeřovská J, Pobišová Z, Bílek R, Hoskovcová P, et al. Relationship between pituitary-thyroid axis hormones and anthropometric parameters in Czech adult population. Physiol Res. 2008;57 Suppl 1:S127-S134. doi: 10.33549/physiolres.931497.
34. Mele C, Mai S, Cena T, Pagano L, Scacchi M, Biondi B, et al. The pattern of TSH and fT4 levels across different BMI ranges in a large cohort of euthyroid patients with obesity. Front Endocrinol (Lausanne). 2022;13:1029376. doi: 10.3389/fendo.2022.1029376.
35. Fox CS, Pencina MJ, D'Agostino RB, Murabito JM, Seely EW, Pearce EN, Vasan RS. Relations of thyroid function to body weight: Cross-sectional and longitudinal observations in a community-based sample. Arch Intern Med. 2008;168:587–92. doi: 10.1001/archinte.168.6.587.
36. Díez JJ, Iglesias P. Relationship between thyrotropin and body mass index in euthyroid subjects. Exp Clin Endocrinol Diabetes. 2011;119:144–50. doi: 10.1055/s-0030-1265133.
37. Makepeace AE, Bremner AP, O'Leary P, Leedman PJ, Feddema P, Michelangeli V, Walsh JP. Significant inverse relationship between serum free T4 concentration and body mass index in euthyroid subjects: Differences between smokers and nonsmokers. Clin Endocrinol (Oxf). 2008;69:648–52. doi: 10.1111/j.1365-2265.2008.03239.x.
38. Manji N, Boelaert K, Sheppard MC, Holder RL, Gough SC, Franklyn JA. Lack of association between serum TSH or free T4 and body mass index in euthyroid subjects. Clin Endocrinol (Oxf). 2006;64:125–8. doi: 10.1111/j.1365-2265.2006.02433.x.
39. Reinehr T. Obesity and thyroid function. Mol Cell Endocrinol. 2010;316:165–71. doi: 10.1016/j.mce.2009.06.005.

40. Hutcheson J. Adipokines influence the inflammatory balance in autoimmunity. Cytokine. 2015;75:272–9. doi: 10.1016/j.cyto.2015.04.004.
41. Fain JN. Release of interleukins and other inflammatory cytokines by human adipose tissue is enhanced in obesity and primarily due to the nonfat cells. Vitam Horm. 2006;74:443–77. doi: 10.1016/S0083-6729(06)74018-3.
42. Nillni EA, Vaslet C, Harris M, Hollenberg A, Bjørbak C, Flier JS. Leptin regulates prothyrotropin-releasing hormone biosynthesis. Evidence for direct and indirect pathways. J Biol Chem. 2000;275:36124–33. doi: 10.1074/jbc.M003549200.
43. Seoane LM, Carro E, Tovar S, Casanueva FF, Dieguez C. Regulation of in vivo TSH secretion by leptin. Regul Pept. 2000;92:25–9. doi: 10.1016/s0167-0115(00)00145-2.
44. Bossowski A, Sawicka B, Szalecki M, Koput A, Wysocka J, Zelazowska-Rutkowska B. Analysis of serum adiponectin, resistin and leptin levels in children and adolescents with autoimmune thyroid disorders. J Pediatr Endocrinol Metab. 2010;23:369–77. doi: 10.1515/jpem.2010.058.

ORIGINAL PAPER

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Botulinum toxin type A in the treatment of laryngeal dystonia: a single-center experience

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ABSTRACT

Aim. The aim of this study was the analysis of the problem of laryngeal dystonia in patients of Otolaryngology and Neurology Clinics to assess the efficiency of effects of Botulin Toxin type A (BOTOX™) injections on voice quality and function of vocal cords with the use of videolaryngostroboscopic and laryngographic examinations, GRBAS (G - grade of hoarseness, R - roughness, B - breathiness, A – aesthenia, S -strain) scale and voice field.

Material and methods. Between 2020 and 2022 a total of 50 patients with laryngeal dystonia were examined. The videolaryngostroboscopic and laryngographic examinations confirmed laryngeal dystonia. Afterwards the patients undertook Botulinum Toxin type A injections into the thyro-arythenoid muscle.

Results. Control examinations confirmed the improvement of vocal folds movability and the decrease in number of irregular vocal folds vibrations. The next injection of BOTOX™ substance was done 3-6 months after the last injection.

Conclusions. The results of our study suggest that although BOTOX™ toxin still remains the main form of treatment for laryngeal dystonia, using it improves phonatory function of larynx (as proven by videolaryngostroboscopic examination).

Introduction

Laryngeal dystonia is a motor dysfunction of laryngeal muscles (1). It is a rarely diagnosed disease, although the first mentions about it came from 1911 when Oppenheim has described the symptoms of variable muscle tension in 4 of his patients and has named it as ''dystonia musculorum deformans". Subsequently Flatten and Sterling (31) introduced a different name of this illness- now introduced as ''progressing torsion spasm". Finally in 1984 the current definition of dystonia was created. It reads as follows: ''laryngeal dystonia is a symptom complex consisting of persisting agonist and antagonist laryngeal muscles, which in w zajętych parts of body cause repeatable and torsional movements. In effect it leads to an abnormal position of group of muscles or even the whole organ against each other or other parts of body." Moreover, the definition of dystonia was modified in XXI century by additng two axis: one focusing on characteristics of clinical feautures and an axis describing the etiology of the disease. These 2 axis decribe the clinical distinguishing features and the strict description of disease's etiology. The modern evaluation and definition of dystonia came as the effect of experience of veterinary medicine, where mutation-caused dystonia-specific brain changes were observed in animals during clinical tests It is belived nowadays that dystonia can be divided into two subgroups due to the etiology: primary (with disorders located in the central nervous system area- can be inherited or acquired) and idiopathic (of unknown cause which occurs not only sporadically but also sometimes is family-related). (2) In primary dystonia the absence of changes in diagnostic imagining (magnetic rezonance, computed tomography) is observed, as well as the ability of nervous system to morphological and biochemical change of neural pathways in response to environmental factors (1). Group of secondary dystonia consists of dystonia plus syndromes, dystonia in the course of degenerative diseases and Parkinson-related dystonia. Dystonia plus syndromes are not related to neurodegenerative diseases, it is worth to point out that other symptoms may be present e.g. ataxia, myoclonus (12)

Dystonia can be classified due to the location into generic dystonia, hemidystonia, segmental dystonia, focal and multifocal dystonia. Focal dystonia of laryngal muscles is rarely diagnosed and it is estimated that it occurs in 1-5,9 / 100 000 inhabitants of the Federal Republic of Germany (4). Currently there is no epidemiological data regarding the occurence of this disease in the territory of Poland. In animals the dystonia is less often diagnosed than in humans. There are some reports of generalized involuntary muscles contractions of unknown etiology in horses, which were discovered in 1910 (5). It is suspected that this disease is analogical to dystonia occuring in humans. Focal dystonia is revealed during implementing specific tasks (as so called task-oriented dystonia), in stress situations or as a result of external stimulus f.e.x light or noise. In humans the laryngeal dystonia is the 3rd most common primary focal dystonia. It occurs more often in women and the early onset of the disease is between 40-50 years old patients. Moreover in 12% the positive family history is confirmed. Most often it occurs in isolated form but in some percentage of patients it occurs in muscles of distant organs. (4, 6). Due to the unknown etiology the risk factors were introduced, which include age more than 40-50 years and positive family history, as well as different forms of stress, chronic inflammations of upper respiratory tract, intensive voice usage, torticollis, late pregnancy and confinement (7).

Koufman divides laryngeal dystonia into 4 main types on the basis of localization of pathological muscle movements (24):

- adductive,
- abductive,
- mixed,
- respiratory.

The most common type of laryngeal dystonia is adductor dystonia – in almost 80% of all patients with this disease. It can be divided into glottic type (when only muscle work is abnormal) and supraglottic type (when abnormal work of vestibular folds and supraglottic muscles are abnormal, as well as tremor of voice is observed). In case of mixed type the division relies on determination of the dominant pathology (abnormal adduction or abduction). Respiratory type is the most rare. The most characteristic feauture for this type are irregular vibations in laryngeal, pharyngeal and palatal muscles present during breathing (which are not present during speak-

ing and sleep). Due to the paradoxal movements of vocal folds stridor occurs as well as abnormal breaks during breathing (8,10, 9).

Aim

The aim of this study was the analysis of the problem of laryngeal dystonia in patients of Otolaryngology and Neurology Clinics of Cracow. The study focued on the assesment of the efficiency of effects of Botulinum Toxin type A injections on voice quality and function of vocal cords with the use of videolaryngostroboscopic examination, laryngography, GRBAS scale and voice field based on the experience of Otolaryngology and Neurology Clinics of the Universty Hospital of Cracow. We also compared our experience with other clinics.

Material and methods

Between 2020 and 2022 a total of 50 patients (46 women and 4 men) with symptoms of laryngeal dystonia have been treated in Otolaryngology and Neurology Clinics of Collegium Medicum of Jagiellonian University in Cracow, Poland. The mean age of examined patients was 67 years (median 76 years), while the youngest patient was 23 years old and the oldest was 89 years old. The medical diagnosis was made after comprehensive neurological examinations, excluding other diseases (e.g. miastenia) and after confirmation in laryngological and phoniatric examination. All patients were qualified for present study. In otolaryngological and phoniatric analysis the GRBAS scale (32) was used for subjective voice evaluation, voice field for sound ''a" was done and the videolaryngostroboscopic and laryngographic examinations were performed. This includes 5 components:

- **G** (grade – an overall grade of hoarseness),
- **R** (roughness),
- **B** (breathiness),
- **A** (aesthenia – weakness),
- **S** (strain).

Each component is rated on four point scale (**0** - normal, **1** - slight, **2** - moderate,and **3** – severe). The parameters of GRBAS scale were used for description of characteristic features of dystonic voice, obtaining the voice field enabled the graphical representation of dependence of individual tones from frequency range to intensity (from minimal to maximal intensity with witch they are created) (25), videolaryngostroboscopic examination was performed in order to asses the local condition as well as the movements of the vocal folds – the mobility and regularity of vocal cords were assesed. The last examination (the laryngography) was used to determinate the percentage share of irregular vocal folds vibrations (expressed as CFx - vocal folds irregularity coefficient). Typically in healthy patients CFx results should be lower than 10-12%; which corresponds to insignificant irregular vocal folds vibrations. In most of the examined patients the adductive type of dystonia was found (45 patients), while the respiratory type occured in 4 patients and 1 patient presented mixed type of dystonia. In every patient of this study botulinum toxin type-A (BOTOX^{tm}) injections into thyro-arytenoid muscle (through cricothyroid membrane) were used as a treatment. It was decided that every first injection will be done on the right side, while the following injections will be done by turns. All injections were guided by the EMG (electromiography) device. Moreover, every participant of the study was informed about possible side effects of botulinum toxin A (e.g. dyspnea). In case of occurence of acute shortness of breath there is a risk of urgent action in form of intubation or performing either the cricothyroidotomy or treacheotomy. After being informed about aboutsaid risks patients with mixed and respiratory types of dystonia refused the constent to treatment with BOTOX^{tm} and as a result this material was not included in this study. The dose of 7.5 units of BOTOX^{tm} was applied to each side which is in line with other studies. The follow-up examinations (otolaryngological-phoniatric) were done respectively 4 and 12 weeks after end of treatment with botululinum toxin. Besides phoniatric-neurological care the patients undertook neurologopedic rehabilitaion (e.g. lax-vox exercises).

Results and analysis

Before the study we observed the high values of R and S parameters of the GRBAS scale. In the analysis the values of G parameter were elevat-

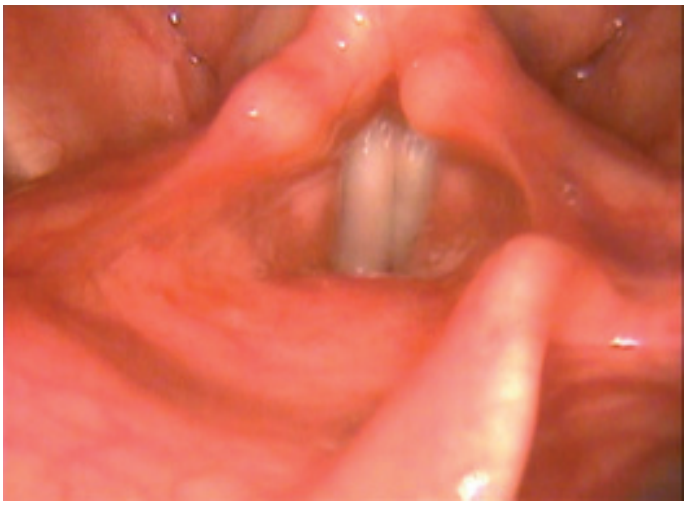

Figure 1. The patient 4 weeks after injection of botulinum toxin type into the right vocal fold area (phonatory position)

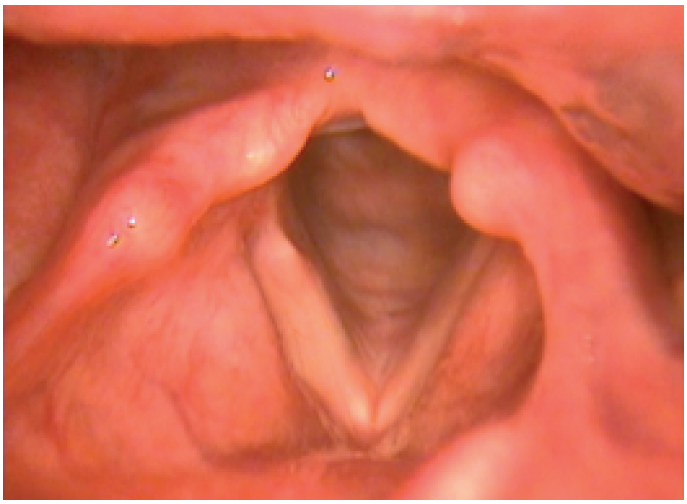

Figure 2. The patient 4 weeks after injection of botulinum toxin type into the right vocal fold area (respiratory position)

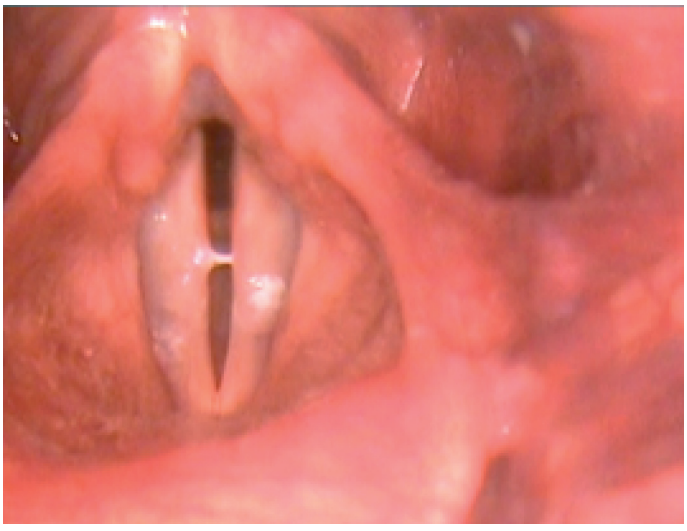

Figure 3. Patient qualified for botulinum toxin type A treatment (inspiratory phase)

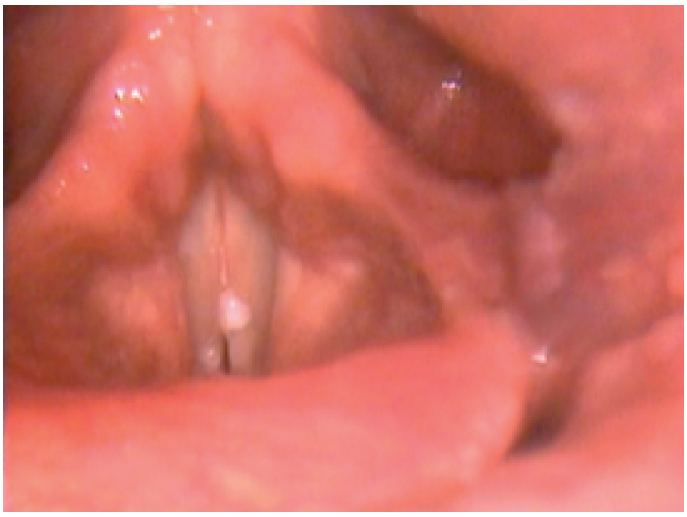

Figure 4. Patient qualified for botulinum toxin type A treatment (phonatory phase)

ed to 1 (22 patients; 48,89%) and 2 (23 patients, 51,11%) in all patients, while the values of R and S parameters were in most cases 3 or in selected cases 2 (e.g. the value of S parameter was as follows: 2 – 17 patients; 37,78% and 3 – 28 patients; 62,22%). The results translates into followings: 0 – *normal, physiological voice*, 1 – *mild changes of voice*; 2 – *average changes of voice*; 3 – *major changes of voice, very severe*. The improvement in movability of vocal folds in terms of regularity of vibrations and phonatory contraction was observed in GRBAS scale results (with simultaneous decrease of hyperfunction of vestibular folds); e.g. in case of G parameter after the therapy the results were as follows: 0 – 10 patients; 22,22%, 1 - 57,78% and 2 – 9 patients; 20%. These results correspond to the observations in the control videolaryngostroboscopy, where in all patients an improvement in mobility and regularity of vocal cords movements was observed. In

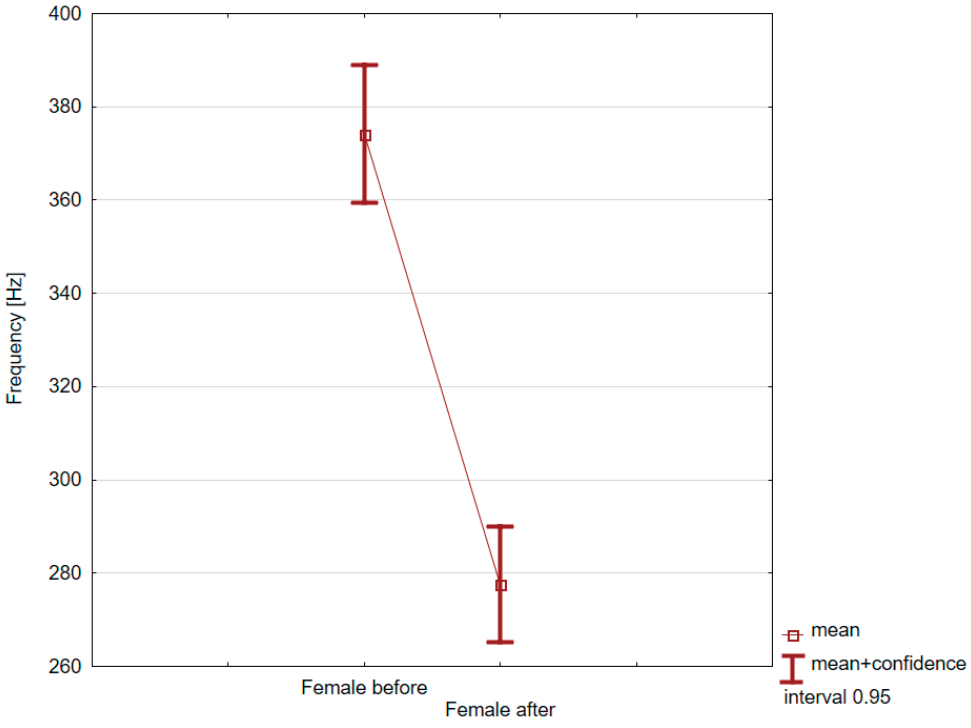

Figure 5. The change of Fmean in female population before and after BOTOXtm treatment (p=0.05).

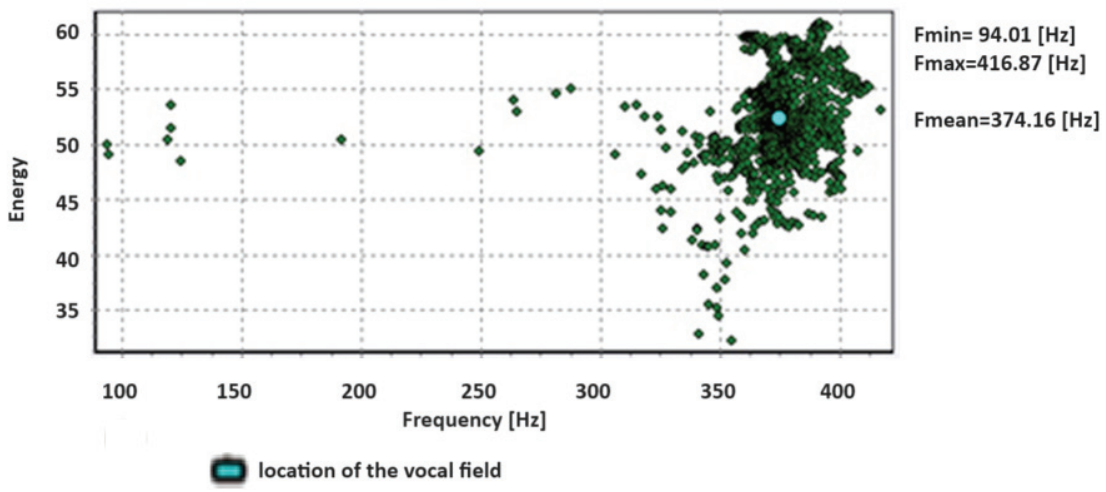

Figure 6. Average results of voice field measurement in females before botulinum toxin type A injection (Hz- frequency; lower bar – the location of the voice field)

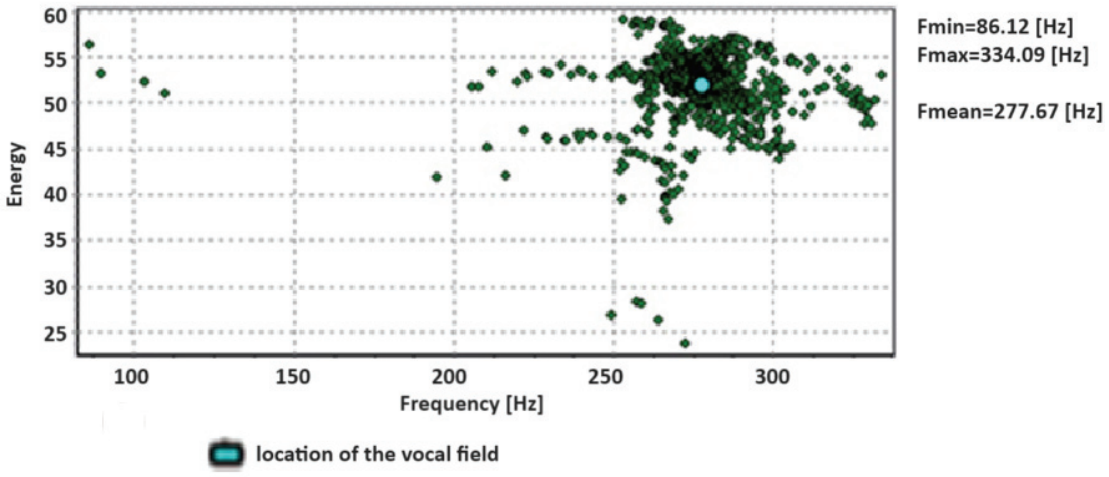

Figure 7. Average result of voice field measurement in females after botulinum toxin type A injection (Hz- frequency; lower bar – the location of the voice field)

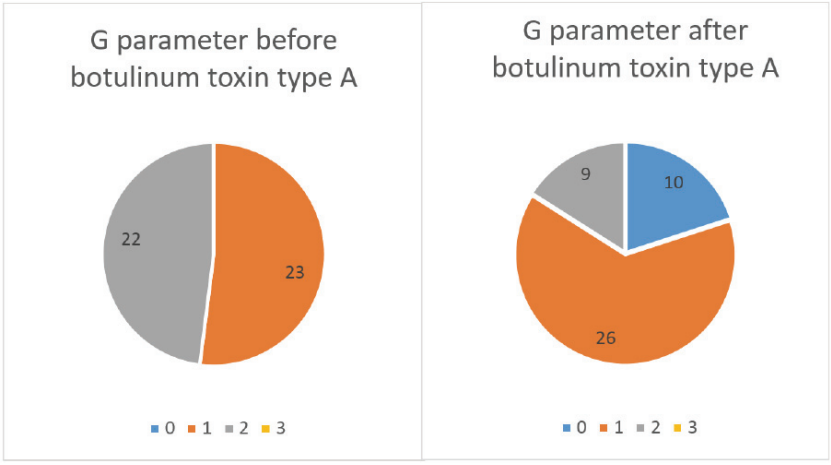

Figure 8. Pie graph showing the distribution of values of G parameter (0-3) of GRBAS scale among examined group of patients before and after botulinum toxin type A injection.

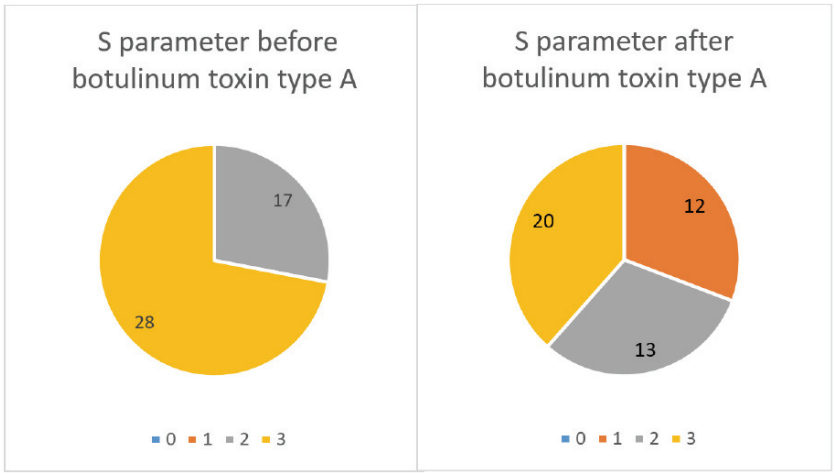

Figure 9. Pie graph showing the distribution of values of S parameter (0-3) of GRBAS scale among examined group of patients before and after botulinum toxin type A injection.

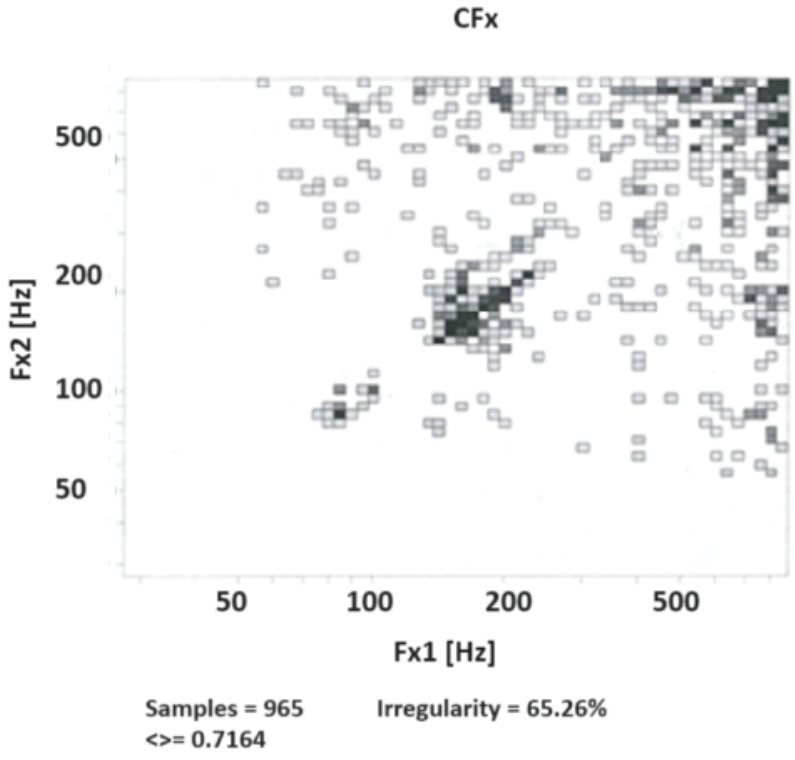

Figure 10. CFx control histogram before botulinum toxin type A therapy with coefficient of irregularity of vocal folds vibrations = 65,26% (p=0.05)

videolaryngostroboscopy before the study the irregular vocal folds vibrations with low mobility were observed in all patients, as well features of as so-called "laryngeal stuttering". The observations in case of this examination may be subjective (depending on the experience of the clinician); due to this fact other controls were performed (e.g. GRBAS scale or laryngographic examination). Before the injection of BOTOX^{tm} the average values for men were as follow: Fmin = 41,50 Hz, Fmax = 806,40 Hz and Fmean = 150,19 Hz, while for females were as follow: Fmin=94,01 Hz, Fmax = 416,87 Hz and Fmean = 374,16 Hz. After the therapy with botulinum toxin type A the following average results were obtained for Fmin, Fmax and Fmean, respectively: 58,98 Hz, 505,63 Hz and 143,00 Hz in males and 86,12 Hz, 334,12 Hz and 277,67 Hz in females. Despite observed algebra-

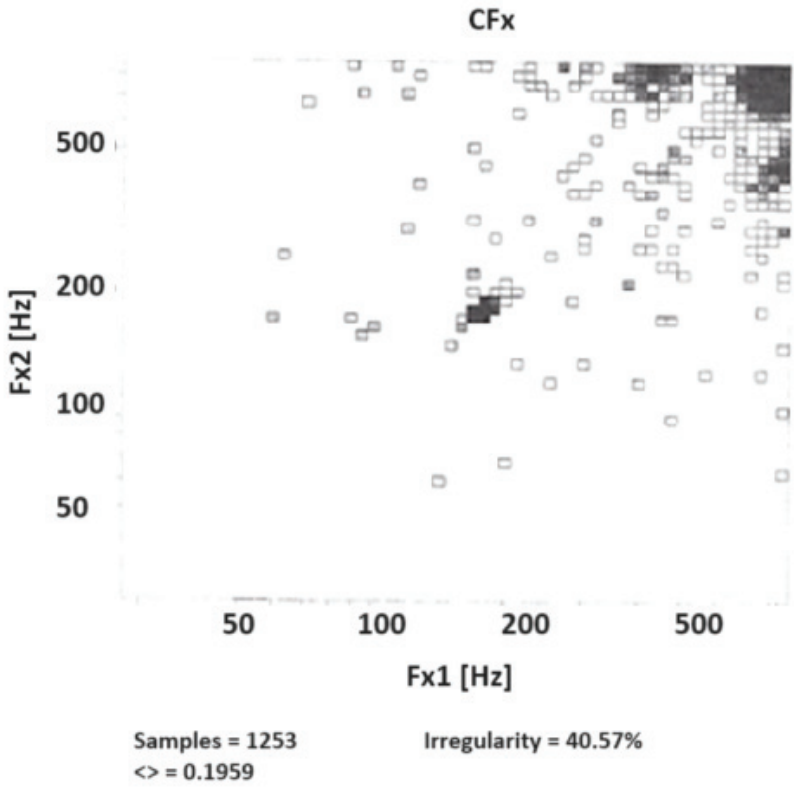

Figure 11. CFx control histogram 4 weeks after the end of botulinum toxin type A therapy with coefficient of irregularity of vocal folds vibrations = 40,57%. (p=0.05)

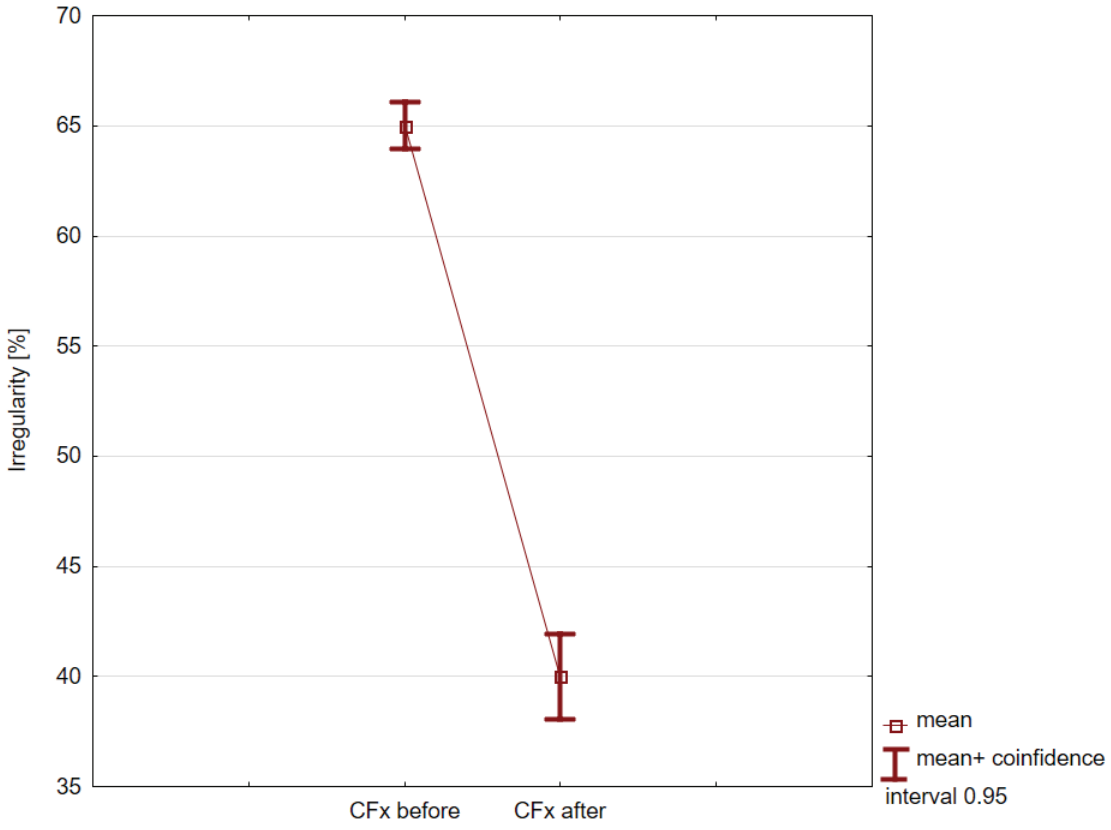

Figure 12. The comparison of irregularity values before and after the BOTOXtm treatment (p=0.05).

ic changes no statistically significant changes were detected in change of Fmean parameter for 2 populations (p=0.05). The high dispersion of measurement value for each patient versus mean value may be the reason behind such results. Therefore it may be challenging to obtain the statistically significant results. The next study should focus on bigger number of patients.

Nevertheless we observed that the change of Fmean in females is bigger than in males. Moreover there is a significant statistical relationship between the decrease of Fmean value (p=0.05) and the clinical improvement of laryngeal function after BOTOX^{tm} administration. The changes of Fmean results for female population were presented in Fig5.

In laryngographic control examinations (performed approximately 4 weeks after the end of therapy) we observed the decrease of value of CFx coefficient to about 45±5% from initial value of more than 60 % in all patients – mean 65±5 (Normal value of CFx should be between 0 and 10/12%) and the results were statistically significant (p=0.05) (Fig. 12).In addition, the lower the value of coefficient the bigger share of regular vocal folds vibrations in phonation process, what itself is the aim of the botulinum toxin therapy. In the following laryngographic examination, done 12 weeks after the first dose of toxin, the values of results increased slighty to CFx level of 50-58%. In case of voice field measurement after the toxin injection the decrease of minimal (Fmin), maximal (Fmax) as well as avarage (Fmean) voice frequency was observed.

The results suggested the stabilization of voice as a result of applied treatment. After 12 weeks the second injection of BOTOX^{tm} was applied into thyro-arytenoid muscles on the opposite site of the first iniection site. In control examination we obtained the similar results.

Discussion

Laryngeal dystonia is a serious neurological and otolaryngological problem and is still being explored and studied by clinicians from across the world. The described above symptoms of respiratory distorders with presence of paradoxal vocal folds vibrations are commonly observed. (30) Animal studies play a big part in an improvement of dystonia diagnostics. It is worth to obtain information about laryngeal dystonia based on animal models because of the similarity between anatomy of larynx in humans and other mammalian species. (18) Despite similarity in mentioned above anatomy and physiology of larynx, partial or complete paralysis of laryngeal muscles occurs more often in animals in comparison to humans (idiopatic paralysis of left recurrent laryngeal nerve in horses). The studies were also performed in dogs, in which laryngeal dystonia was induced after performing tracheotomy and connecting Harvard electrodes with recurrent laryngeal nerve (19). Stimulation of laryngeal muscles was used (cricothyroid and crico-arytenoid) and subsequently the impact of excessive muscles contraction on intraglottal pressure was investigated. Arytenoid cartilages were sustained in imposed, constant adduction as a result of this stimulation. The aim of these studies was to compare intraglottal pressure before and after stimulation of mentioned above muscles, which confirmed the impact of excessive muscle spasm on obtained results.

The current recommended method for laryngeal dystonia treatment (as well as for other focal dystonias) is the injection of botulinum toxin (BTX) into affected muscles (26). It is one of the strongest known neurotoxins of natural origin. Lethal dose for human is 0,2-2,0 μg/kg and is exposure-dependent (27). Gram-positive *Clostridium botulinum* is responsible for its production as a result of anaerobic fermentation (27). Currently there are 8 serotypes of botulinum toxin (A-H). The differences between them are as follows: molecular weight, antigenic structure, immunogenicity, receptors (and localisation of genes which are responsible for coding tchem) and half life time (27). In case of laryngeal dystonia type A of botulinum toxin was used, first in 1988 (28). Usually 2,5 units of botox are injected bilaterally or 5 units unilaterally. The effects of using the toxin appear about 3-7 days after the injection, when BTX binds with SV2C receptor and as a result SNARE protein is being blocked. Novakovich and associates (20, 27) report that even 28,5% of examined patients reported initial decrease of function of voice after administration of the toxin. Such effect is maintained for about 2 weeks, while during the next 6 weeks the normalization of voice occurs and the patient notices an

improvement in speech. After this time during the next 4 weeks the gradual worsening of phonation occurs. Moreover he reports that the disorders of phonation may be a result of f.ex wrong administration technique or drug dose. The resistance to botulinum toxin iniection is possible too. Marshall E. Smith and Charles N. Ford report 2 cases, where after long-term application of botulinum toxin the resistance to this substance and lack of effect after injection occured as a result. However, this phenomenon rarely occurs but in order to prevent it from happening the administration of the lowest effective dose and maintaining minimum 3 month-breaks between subsequent injections are advised. (33) The experience of University Hospital of Cracow in using botulinum neurotoxin is in line with other researchers as it prevails as the standard of care in LD, especially with a large body of evidence attesting to its efficacy. In Japan Hirose et al. demonstrated its therapeutic efficacy through a placebo-controlled, randomised, double-blind clinical trial. Botulinum Toxin therapy has been accepted and funded by the Japanese medical insurance scheme as a treatment for LD as a result of mentioned above study. Along with the work of Hyodo et al. (34) Dongren Yao et al. point that botulinum toxin injections are considered the first line of therapy of dystonia, with the highest use of botulinum toxin is reported in laryngeal dystonia and blepharospasm .(35) Moreover, Robert J. Stachler et al. in the United States recommend using botulinum toxin therapy along with regular laryngoscopy and prior to voice therapy in treatment of LD, what is in line with the experience of the Otolaryngology Clinic of Cracow. (36)

Currently there are no effective alternatives to therapy using injections of botulinum toxin type A. Berke and associates (21) have proposed surgical treatment of patients with adductive type of laryngeal dystonia. It is based on selective denervating and reinnervation of muscles responsible for adductive function of larynx - initially unilaterally, finally bilaterally. Even though the short-term results had brought hope for an improvment of phonatory function of larynx, the phonatory disorders returned in the long-term time. Thyroplasty type II is another surgical method and was introduced by Isshiki and associates (28). The aim of this method was prevention of excessive closure of glottis during phonation as a result of abnormal contractions. Failures of using above method in treatment of LD may be the result of difficulties in performing such type of surgery. Moreover, despite attempts with oral pharmacotherapy (e.g. anticholinergic drugs) no drug has been registered for laryngeal dystonia treatment. Pharmacotherapy itself is allowed as supportive therapy for treatment of patients with botulinum toxin type A. Speech therapy may play a role in treatment of laryngeal dystonia but currently there are no results of clinical studies confirming its effectiveness (22). Neurologopedic therapy can be an important part of the treatment due to the necessity of rehabilitation of communicative proces (which is disturbed to a large extent as a result of laryngeal dystonia).

Furthermore among new methods of treatment of laryngeal dystonia 2 groups are mentioned: central nervous system-focused methods and larynx-oriented therapies. First group includes e.g. deep brain stimulation. It is used in neurological disorders, like Parkinson disease; but in case of laryngeal dystonia using it requies special permisson. DEBUSSY (Thalamic Deep Brain Stimulation for Spasmodic Dysphonia) trial is an example of using deep brain stimulation in treatment of LD. In case of receiving positive results of the expermient this method may be used in patients with laryngeal dystonia. Second group consists of so called vibrostimulation of larynx and neuromodulation of larynx as a result of electrostimulation. The first of mentioned aboved methods is based on treating somatosensory dysfunction of the phonatory organ with usage of non-invasive vibrostimulation of lateral surfaces of thyroid cartilage (time duration: 40 minutes). In analysis after the stimulation the effect was mantained for about 20 minutes in 69% of examined patients. Currently studies are underway with aim of finding of optimal level of stimulation for obtaining the best effects of the treatment (29, 28). Neuromodulation of larynx is the second method. in the conducted study the stimulation of left thyroarytenoid muscles through hooked electrode was done. It was carried out for 5 days, 1 hour a day on a level lower than required for motor neuron activation. In 4 out of 5 patients the improvement in phonatory function was observed after the end of therapy and it lasted maximum of 14 days. Currently the new studies are underway with bigger number of examined patients. (28).

Conculsions

Laryngeal dystonia is an example of a disease, where the interdisciplinary cooperation is necessary. Videolaryngostroboscopic examination plays the key role in diagnostics of the disease. Non-invasive visualization of speech apparatus (phonatory) and determination of degree of voice disorders are both possible by using the laryngographic examination. GRBAS scale, as well as voice field measurement are another examples of useful, non-invasive phoniatric methods, which can be used to measure progress for both botulinum toxin type A therapy, as well as neurologopedic rehabilitation. The clinical results of our study suggest that botulinum toxin A injections improve phonatory function of the larynx, which provides an argument for its continuous use for layngeal dystonia.

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Authors contribution
Remigiusz Ziarno (conceptualization, data curation, formal analysis, project administration, writing – original draft); Małgorzata Dec-Ćwiek (data collection, analysis); Magdalena Sobuś (analysis, writing of the paper); Konrad Skórkiewicz (analysis, writing of the paper); Aleksandra Grudzień-Ziarno (analysis, writing of the paper); Jacek Składzień (data collection, analysis); Jerzy Tomik (data collection, writing of the paper, analysis)

Ethical standards
The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guidelines on human experimentation (please name) and with the Helsinki Declaration of 1975, as revised in 2008 and assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides.

Conflict of interest statement
The authors declare no conflict of interest.

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References

1. Richter A, Hamann M, Wissel J, Volk HA: Dystonia and Paroxysmal Dyskinesias: Under-Recognized Movement Disorders in Domestic Animals? A Comparison with Human Dystonia/Paroxysmal Dyskinesias. Front Vet Sci, 2015 Nov 30;2:65. doi: 10.3389/fvets.2015.00065.
2. Albanese A, Bhatia K, Bressman SB, DeLong MR, Fahn S, Fung VSC i wsp: Phenomenology and Classification of Dystonia: A Consensus Update. Movement Disorders, 2013 Jun 15;28(7):863-73. doi: 10.1002/mds.25475.
3. Evinger C: Animal models of focal dystonia. NeuroRx. 2005 Jul;2:513-24. doi: 10.1602/neurorx.2.3.513.
4. Castelon Konkiewitz E, Trender-Gerhard I, Kamm C, Warner T, Ben-Shlomo Y, Gasser T, Conrad B, Ceballos-Baumann AO: Service-based survey of dystonia in Munich. Neuroepidemiology. 2002 Jul-Aug;21:202-6. doi: 10.1159/000059525.
5. McCall Jr., Stringhalt" and shivering. Proceedings 28th General Meeting of the National Veterinary Association (1910): 23–56. doi: 10.1016/j.tvjl.2022.105829.
6. Blitzer A: Spasmodic dysphonia and botulinum toxin: experience from the largest treatment series. Eur J Neurol, 2010 Jul;17 Suppl 1:28-30. doi: 10.1111/j.1468-1331.2010.03047.x.
7. Blitzer A, Brin MF, Simonyan K., Ozelius LJ, Frucht SJ: Phenomenology, Genetics and CNS network abnormalities in Laryngeal Dystonia: a 30 year experience. Laryngoscope, 2018;128 Suppl 1:S1–S9. doi: 10.1002/lary.27003. Epub 2017 Dec 8.
8. Santos VJB, Mattioli FM, Mattioli WM, Daniel RJ, Vicente Paulo Miranda Cruz VPM: Laryngeal dystonia: case report and treatment with botulinum toxin. Rev Bras Otorrinolaringol 2006;72:425-7. doi: 10.1016/s1808-8694(15)30980-0.
9. Janczewski G: Otorynolaryngologia praktyczna, podręcznik dla studentów i lekarzy, tom II pod redakcją Grzegorza Janczewskiego. Via Medica, Gdańsk 2005, wyd. 1.
10. De Biase NG; De Lima Pontes PA; Santos Junior V; Vieira VP; Zambonato P; Yazaki RK: The difficult management of patients with respiratory segmental dystonia. Braz J Otorhinolaryngol, 2007 Mar-Apr;73:278-83. doi: 10.1016/s1808-8694(15)31078-8.
11. Zbrojkiewicz M, Lebiedowska A, Błońska-Fajfrowska B: Toksyna botulinowa w medycynie i kosmetologii – dwustuletnia historia i nowe perspektywy. Postepy Hig Med Dosw (online), 2018; 72: 278-289. e-ISSN 1732-2693.
12. Kartha N: Wyzwania terapeutyczne w leczeniu dystonii. Neurologia po Dyplomie, Tom 5 Nr 6 2010, 45-54.
13. Meyer-Lindberg A., Wohlfarth KM, Switzer EN. The use of botulinum toxin A for treatment of possible essential blepharospasm in a dog. Aust Vet J (2003) 81: 612–4. doi: 10.1111/j.1751-0813.2003.tb12503.x.
14. Namin AW, Christopher KM, Eisenbeis JF: Botulinum Toxin Dosing Trends in Spasmodic Dysphonia Over a 20-year Period. J Voice, 2017 Jan;31:107-110. doi: 10.1016/j.jvoice.2016.01.006. .
15. Upile T, Elmiyeh B, Jerjes W, Prasad V, Kafas P, Abiola J, Youl B, Epstein R, Hopper C, Sudhoff H, Rubin J. Unilateral versus bilateral thyroarytenoid Botulinum toxin injections in adductor spasmodic dysphonia: a prospective study. Head Face Med. 2009 Oct 24;5:20. doi: 10.1186/1746-160X-5-20.
16. Halstead LA, McBroom DM, Bonilha HS: Task-specific singing dystonia: vocal instability that technique cannot fix. J Voice. 2015 Jan;29:71-8. Epub 2014 Jul 5. doi: 10.1016/j.jvoice.2014.04.011.

17. Payne S, Tisch S, Cole I, Brake H, Rough J, Darveniza P: The clinical spectrum of laryngeal dystonia includes dystonic cough: observations of a large series. Mov Disord. 2014 May; 29:729-35. doi: 10.1002/mds.25865.
18. Cook WR: A comparison of idiopathic laryngeal paralysis in man and horse. J Laryngol Otol, 1970 Aug;84:819-35
19. Green DC, Berke GS. An in vivo canine model for testing treatment effects in laryngeal hyperadduction disorders. Laryngoscope. 1990 Nov;100(11):1229-35. doi: 10.1288/00005537-199011000-00019.
20. Novakovic D, Waters HH, D'Elia JB, Blitzer A. Botulinum toxin treatment of adductor spasmodic dysphonia: longitudinal functional outcomes. Laryngoscope. 2011 Mar;121(3):606-12. doi: 10.1002/lary.21395.
21. Berke GS, Blackwell KE, Gerratt BR, Verneil A, Jackson KS, Sercarz JA. Selective laryngeal adductor denervation-reinnervation: A new surgical treatment for adductor spasmodic dysphonia. Ann Otol Rhinol Laryngol 1999;108: 227–231. doi: 10.1177/000348949910800302.
22. Mor N, Blitzer A: Diagnosis and Treatment of Laryngeal Dystonia: Past, Present and Future Directions. Tremor and Other Hyperkinetic Movements 2016.
23. Stewart CF, Sinclair CF, Kling IF, Diamond BE, Blitzer A: Adductor focal laryngeal Dystonia: correlation between clinicians' ratings and subjects' perception of dysphonia. Stewart et al. Journal of Clinical Movement Disorders (2017) 4:20. doi: 10.1186/s40734-017-0066-y.
24. Koufman JA, Blalock PD. Classification of laryngeal dystonias - Center for Voice Disorders of Wake Forest University 2003.
25. Niebudek-Bogusz E i wsp. Rehabilitacja zawodowych zaburzeń głosu. Poradnik dla nauczycieli. Publikacja współfinansowana przez Unię Europejską w ramach Europejskiego Funduszu Społecznego. Instytut Medycyny Pracy im. prof. J. Nofera. Łódź 2009.
26. Niebudek-Bogusz E, Modzelewska-Radwan B, Pietruszewska W, Zielińska M, Gryczyński M. Trudności diagnostyczne i terapeutyczne w dystoniach krtaniowych. Otolaryngologia Polska, 2003;57:739-745.
27. Zbrojkiewicz M, Lebiedowska A, Błońska-Fajfrowska B. Toksyna botulinowa w medycynie i kosmetologii – dwustuletnia historia i nowe perspektywy. Postepy Hig Med Dosw (online), 2018; 72: 278-289.
28. Enver N, Pitman MJ. What Is New in Laryngeal Dystonia: Review of Novel Findings of Pathophysiology and Novel Treatment Options. Curr Otorhinolaryngol Rep 8, 209–215 (2020). https://doi.org/10.1007/s40136-020-00301-x.
29. Khosravani, S., Mahnan, A., Yeh, IL. et al. Laryngeal vibration as a non-invasive neuromodulation therapy for spasmodic dysphonia. Sci Rep 9, 17955 (2019). doi: 10.1038/s41598-019-54396-4.
30. Tierney WS, Bryson PC, Nelson R, Kaplan SE, Benninger MS, Milstein CF. Respiratory Laryngeal Dystonia: Characterization and Diagnosis of a Rare Neurogenic Disorder. Laryngoscope. 2020 Dec;130:2843-2846, Epub 2020 Feb 19. doi: 10.1002/lary.28559.
31. Flatau E., Sterling W. Progressiver Torsionspasms bei Kindern. Zeitschrift für die Gesamte Neurologie und Psychiatrie 7, 586–612, 1911.
32. Hirano, M. Clinical examination of voice. Wien-New-York: Springer-Verlag, 1981.
33. Smith M.E., Ford Ch.N. Resistance to Botulinum Toxin Injections for Spasmoid Dysphonia. Arch Otolaryngol Head Neck Surg/Vol 126, Apr 2000. doi: 10.1001/archotol.126.4.533.
34. Yeung W, Richards AL, Novakovic D. Botulinum Neurotoxin Therapy in the Clinical Management of Laryngeal Dystonia. Toxins (Basel). 2022 Dec 1;14(12):844. doi: 10.3390/toxins14120844.
35. Yao D, O'Flynn LC, Simonyan K. DystoniaBoTXNet: Novel Neural Network Biomarker of Botulinum Toxin Efficacy in Isolated Dystonia. Ann Neurol. 2023 Mar;93(3):460-471. doi: 10.1002/ana.26558.
36. Stachler RJ, Francis DO, Schwartz SR, Damask CC, Digoy GP, Krouse HJ, McCoy SJ, Ouellette DR, Patel RR, Reavis CCW, Smith LJ, Smith M, Strode SW, Woo P, Nnacheta LC. Clinical Practice Guideline: Hoarseness (Dysphonia) (Update) Executive Summary. Otolaryngol Head Neck Surg. 2018 Mar;158(3):409-426. doi: 10.1177/0194599817751031. Erratum in: Otolaryngol Head Neck Surg. 2018 Aug;159(2):403.

REVIEW PAPER

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Stridor in pediatrics is not only laryngomalacia

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ABSTRACT

Introduction. Stridor in newborns and infants is a common manifestation of airway obstruction that can be caused by both benign and severe pathologies.
Aim. This literature review examines the diseases that can present with stridor in children after birth, with the aim of better understanding their etiology, clinical course, and treatment.
Material and methods. The literature reviewed included published studies, reports, guidelines, and consensus statements on pediatric laryngeal pathologies associated with stridor.
Results. Laryngomalacia is the leading cause of stridor (up to 75% of cases) with a mostly mild course that requires only observation. However, stridor may also indicate other, often more serious laryngeal pathologies, such as vocal cord paralysis, subglottic stenosis, laryngeal cleft, and subglottic haemangioma, usually requiring surgical intervention.
Conclusions. Stridor is not a specific symptom of only laryngomalacia. Laryngeal pathologies manifested as stridor can have different etiology and, as a result, require a fundamentally different approach to treatment.

Introduction

Stridor is included as an entity in the International Classification of Diseases (R06.1 in ICD 10 and MD11.B in ICD 11), but it is only a symptom, not a diagnosis. It is described as noisy breathing that occurs due to obstruction at different segments of the airway. Due to the areas of obstruction in the airways of the child, a turbulent airflow is formed, which causes a characteristic whistling noise while breathing. Depending on the level of airway obstruction, the stridor can appear both in the inspiratory and the expiratory stages, and it can be biphasic in case of pathology at the level of the vocal fold, subglottis, or upper trachea areas. To make a correct diagnosis, it is important

to know the anatomy and understanding of the nature of the stridor, a thorough anamnesis, and of course, modern airway examination capabilities and methods. Voice condition also provides additional information to understand the level of obstruction and possible diagnosis. Hoarseness of the voice usually indicates an obstruction at the level of the vocal folds. A muffled voice is usually a symptom of processes at the level of the epiglottis. Stridor with congenital diseases can develop from the first hours of a child's life, as well as during the first days, weeks, or even months after birth. Patients of different age groups often have different congenital and acquired conditions that can manifest as stridor, which are important to recognize. A thorough examination of the patient, collecting anamnesis, and a deep understanding of the nature of the stridor will help to establish a preliminary diagnosis of the patient and provide an algorithm of actions for further invasive diagnostics and evaluation of the possibilities of conservative and surgical treatment of such patients. The authors aim to review the most common causes of stridor in children as well as modern approaches to the diagnosis and treatment of these conditions in pediatric patients.

Laryngomalacia

Laryngomalacia is the most common congenital laryngeal disease in children and reaches up to 75% of all stridor cases according to the literature [1,2]. Laryngomalacia is characterized by the collapse of the supraglottic structures during inspiration, which leads to a spurting airflow and high inspiratory stridor typical for the disease. In children with congenital laryngomalacia, stridor does not manifest immediately after birth, but rather a more typical progression of symptoms and the appearance of an obvious stridor at the age of several weeks. Symptoms may become more severe during the first 4–8 months of life. The stridor worsens with the baby's supine position, when the baby is nervous, during the crying and feeding process, and with upper respiratory tract infections. Approximately half of children with mild to moderate laryngomalacia will have feeding problems and problems in addition to respiratory symptoms. However, almost all children with mild laryngomalacia have feeding difficulties, which may be associated with episodes of cyanosis, cough, and choking. In children with severe disease, there is a lag in the rate of weight gain due to feeding disorders, which may be accompanied by aspiration pneumonia. Such patients have an unpredictable amount of calories consumed due to more frequent feeding and increased metabolic demand due to elevated respiratory work [3]. In the literature, there are usually three different types of laryngomalacia characterized by prolapse of excess mucosa into the lumen of the larynx, a combination of shortened scooped epiglottis and twisted omega epiglottis, a mobile epiglottis that collapses into the larynx during breathing [4].

Historically, laryngomalacia was considered to be a congenital anatomical pathology, but modern theories relate the disease more to a neuromuscular etiology, which is based on the failure of the peripheral nerves to maintain the shape of the airway, which ensures its patency and coordinates the act of swallowing in children. Some studies have investigated the threshold of stimulus to induce a normal motor response of the laryngeal structures in children with LM, the results of which were correlated with the severity of the disease [1]. As well as histological studies that revealed differences in the size of the branches of the upper laryngeal nerve in children with LM and healthy children of the same age. The neuromuscular etiology and the possibility of further development of the nervous system of newborns may explain the spontaneous improvement and recovery that is usually observed in children with LM [5].

A preliminary diagnosis of laryngomalacia can be made based on a characteristic anamnesis, symptoms, and a thorough physical examination of the child. The most characteristic of congenital laryngomalacia is the manifestation of symptoms from 2–4 weeks of life, unlike other pathologies that can manifest themselves with stridor from the first hours of life. Inspiratory stridor is characterized by worsening in the supine position, when the child is worried and during crying, in severe cases of the disease, weight lag is characteristic, so it is important to measure the child's height and weight. Feeding disorders may be accompanied by frequent aspiration pneumonia. Respiratory disorders can be accompanied by suffocation and cyanosis, it is important

to examine the child's chest for chest retraction or contraction during the act of inhalation. In most cases, a definitive diagnosis can be made by fibrolaryngoscopy without anesthesia during spontaneous breathing. Although fibrolaryngoscopy is the gold standard for the diagnosis of LM, it still has its limitations, such as the inability to assess structures below the vocal folds. It is also important to remember that the course of stridor and the severity of symptoms do not always have a direct correlation with the identified collapse of laryngeal structures and the severity of this collapse. If concomitant airway pathologies are suspected or if the clinical picture is inconsistent with the results of fibrolaryngoscopy, a deeper laryngotracheoscopy using rigid optics under general anesthesia should be performed. Such a technique makes it possible to visualize the larynx more accurately, unlike fiberoptic examination, which has its limitations in image quality due to the optical fibers. As well as performing endoscopy with rigid optics, it is even possible to examine the airways more completely to exclude comorbidities. There are also recommendations for performing polysomnography in children with severe LM and in children in whom surgical treatment has not given a significant improvement [6].

In children with mild laryngomalacia manifested by only a minor inspiratory stridor, a control examination should be performed once a month, in case of symptom stability or improvement, the interval between control examinations can be increased to 3–6 months. In the case of severe laryngomalacia that is additionally manifested by cough, choking, regurgitation, feeding difficulties, apnea, cyanosis, failure to thrive, pulmonary hypertension, and cor-pulmonale, it is necessary to start treatment with acid suppression therapy and consider feeding therapy and swallowing evaluation as well as laryngotracheoscopy and supraglottoplasty (which is the gold standard of surgical treatment). In the case of multiple severe comorbidities or multilevel airway obstruction not amenable to surgical management, it is necessary to consider tracheostomy [6].

Vocal cord paralysis

Vocal Cord Paresis or Paralysis (VCP) is another common cause of stridor in infants and older children, constituting up to 60% of stridor cases [7]. It is defined as an absence of vocal fold movement and can be bilateral (BVCP) or unilateral (UVCP). VCP is the effect of damaged nerve supply to the muscles of the larynx [8]. In approximately 40% of patients with VCP, another airway disease coexists, and laryngomalacia is the most common [3]. UVCP is more frequent, with a left fold predominance. Around 70% of UVCP cases have an iatrogenic origin, being mainly a result of cardiovascular or thoracic procedures. Prolonged intubation along with neurologic diseases and birth trauma compose approximately 50% of cases of BVCP and 21% are considered idiopathic [9]. In patients with BVCP who did not undergo any surgical procedures, neurologic diseases such as Arnold Chiari malformation or congenital myasthenic syndromes (CMS) need to be suspected, and so, further imaging and a careful neurologic examination is recommended [10]. In patients with Arnold Chiari malformation the protrusion of the brainstem through the foramen magnum is connected with compressing the vagus nerve which in effect contributes to VCP development [11].

Symptoms differ between patients with UVCP and BVCP and are more severe in BVCP. Neonates with BVCP present with a weak cry, dyspnoea, inspiratory stridor, or even cyanosis, whereas in patients with UVCP, a typical sign is dysphonia. In both cases, symptoms intensify upon agitation, although some UVCP might pass unrecognized [12]. Another great concern amongst patients with VCP is the risk of silent aspiration, reaching over 50% according to recent data, making the evaluation of swallowing function necessary [13]. To set the diagnosis in a stable patient a flexible fiberoscopy seems to be a procedure of choice [7].

The main goal of surgery in VCP is to achieve safe airway and feeding at the same time, as many neonates with VCP have concomitant problems with nursing and might require a gastrostomy or nasogastric tube feeding until a "safe swallow" is obtained [9]. The surgical approach depends on the patient's general condition, his comorbidities, especially the risk of aspiration, and spreads from endoscopic procedures such as adenoidectomy, vocal fold cordectomy, fold lateralization to open laryngotracheoplasty with a posterior cartilage graft or in some cases – a tracheostomy [14]. In cases of BVCP where surgical management is

focused on widening the posterior glottic space, the risk of future aspiration and voice disorders increases [15].

When establishing the best moment for surgical intervention, the surgeon should keep in mind that up to 70% of VCP cases recover spontaneously. The rehabilitation of damaged laryngeal nerve is frequently unpredictable and might last up to 12 months, with iatrogenic VFP unfortunately having the lowest percentage of healing. According to that, a patient should be observed for at least a year and, in cases of open surgeries, even up to two years before a surgical procedure is undertaken [7,16].

Congenital subglottic stenosis

Congenital subglottic stenosis (SGS) is usually identified by the authors as the third most common congenital laryngeal malformation after laryngomalacia and vocal fold paralysis [17]. About 30% of all congenital anomalies of the larynx that provoke stridor may be congenital subglottic stenosis [18]. Congenital SGS is defined as a constriction of less than 4 mm for full-term infants and less than 3 mm for preterm infants [19] at the level of the cricoid cartilage with the maximum level of constriction 2–3 mm below the vocal folds in children who had no previous episodes of intubation or endoscopic examinations. Congenital SGS requires evaluation of the larynx with fiberoptic endoscopy for dynamic assessment of the vocal folds and a further endoscopy using rigid optics as the best possible method of visualization and evaluation of the larynx.

Congenital SGC can be divided into two types: a membranous and a cartilaginous type, both of which occur as a result of incomplete recanalization of the laryngeal lumen during embryogenesis. The membranous type is much more common and occurs due to hypertrophy of the submucosa with an excess of fibrous connective tissue and is a milder type of congenital SGS. The cartilaginous type occurs due to an abnormal, usually elliptical, shape of the cricoid cartilage and is a more severe form of congenital SGS that can be classified as grade III-IV stenosis according to the Cotton-Myer grading system [20].

In children with mild subglottic stenosis, manifestations may appear only during recurrent respiratory infections, during which mild edema of the laryngeal mucosa can provoke airway obstruction (recurrent croup). In most cases, such mild forms of subglottic stenosis resolve spontaneously with the patient's growth, in other cases, endoscopic surgery with radial incisions of the membrane and following laryngeal dilation can be performed [21]. More severe cases can manifest as biphasic stridor and even as acute airway compromise during delivery, and such patients may require endotracheal intubation or even an emergency tracheostomy. In the case of endotracheal intubation, the risk of more severe acquired subglottic stenosis increases significantly, which may require open laryngotracheal reconstruction using costal cartilage as a laryngeal graft or cricotracheal resection [22].

Laryngeal webs

Congenital laryngeal webs (LW) constitute 5% of laryngeal congenital defects causing airway obstruction and arise between the 6th and 10th week of embryogenesis [23]. LW can present a wide range of symptoms – from voice disorders – such as hoarseness to severe dyspnea with stridor depending on the grade of larynx lumen involved in the process [24]. Since 1985, LW grading has been based on the Cohen scale, where type 1 are described as thin, membranous webs, occupying less than 35% of the glottis. Type 2 is defined as a web covering up to 50% of glottic space. Type 3 involves not only 50–75% of the glottis, but might spread to the anterior cricoid in the subglottic space. In type 4 – the most severe type – up to 99% of glottis is involved [25]. In cases of complete congenital laryngeal stenosis, a rare and fetal congenital high airway obstruction syndrome (CHAOS) might be suspected, as laryngeal atresia is its most frequent cause [26].

The way of treatment depends on the type of web and symptoms, where type 1 and 2 might not require any surgical intervention if asymptomatic, and remain only under observation. If symptomatic, they are almost always managed endoscopically. Web can be cut with the use of a cold steel laryngeal knife or a CO2 laser. In some cases, the laryngeal keel needs to be fixed in the anterior part of the glottis to inhibit scar-

ring. Types 3 and 4 usually require an emergency tracheostomy in the first days or months of life, before the target surgical approach is taken, which often consists of open laryngoplasty with rib graft placement [24,27].

Cases of type 2 webs give most concerns regarding surgical approach as they present a wide range of both morphological features and in consequence symptoms. They can be thin and membranous or thick with subglottic involvement which would demand different surgical treatments, and the approach must be tailored to a certain case not only to a web type [28].

In patients with laryngeal webs, there is a higher prevalence of other laryngeal abnormalities such as laryngeal subglottic stenosis or trachea-esophageal fistulas [24]. According to recent data, 30% up to 65% of patients with the laryngeal web have 22q11.2 deletion syndrome, commonly called DiGeorge syndrome. It is the most common chromosomal microdeletion and its characteristic features as well as comorbidities should be kept in mind when diagnosing a patient with LW [29].

Subglottic hemangioma

Hemangiomas are benign tumors, appearing in childhood, caused by abnormalities in angiogenesis. They are built up of endothelium cells and some parts of stroma such as fibroblasts, macrophages, and pericytes[30]. They are quite common as they occur in 4–10%of infants and they have a phase of rapid proliferation and involution [31]. Hemangiomas are a challenge in case they are part of some syndrome or are located in the airway tract, narrowing air flow, causing respiratory stridor and other severe breathing disorders.

Hemangiomas have two times higher prevalence among females [32]. They are more common among caucasian infants [33]. Around 60% of infantile hemangiomas are located around the head and neck but in contrast, subglottic hemangiomas (SGH) are infrequent benign tumors of airways and are responsible for 1,5% of congenital laryngeal anomalies [30]. Syndromes connected with hemangioma are hemangiomatosis, PHACE(s), sacral, and PELVIS syndrome.

The typical clinical course in infants with subglottic hemangioma (SGH) is asymptomatic in the early weeks of life. The first symptoms are biphasic stridor, recurrent or prolonged croup, and barking cough occur between 2–4 months. [32]. The basic diagnostic method consists of medical reconnaissance and rigid endoscopy. The most frequent SGH occurs in the left posterior portion of the subglottis. Pink or bluish, submucosal, smooth, round, compressible subglottic mass which leads to asymmetry and stenosis of the airway is a typical view in endoscopy. Diagnosis is based on endoscopic findings. Performing a biopsy is not recommended in this case [30].

Infantile hemangiomas are an interdisciplinary issue. There are a lot of advantages to using propranolol, a nonselective beta blocker. Treatment is initiated usually in the hospital to observe patients for unwanted reactions, at 1 mg/kg of propranolol 3 times a day, then increase to 2 mg/kg and is continued for 6–12 months [34]. Propranolol is considered the best treatment option for SGH nowadays but it is still under evaluation [32]. Response to treatment is confirmed by serial endoscopy. Other alternative treatment methods include intralesional and systemic steroids, CO2 laser resection, microdebrider submucosal resection, or open surgery. All these options have side effects and are considered only in certain cases [31].

Laryngeal cleft

Laryngotracheoesophageal clefts are a group of rare congenital anomalies characterized by the presence of pathological communication between the esophagus and the airway tract and are estimated to occur from 1 in 10,000 newborns to 1 in 20,000 newborns [35].

Classification of laryngeal clefts proposed by Benjamin and Inglis in 1989 remains the most popular and functional and identifies 4 main types [36]: Type I is a supraglottic intraaratenoid defect above the level of the true vocal folds; Type II includes a partial defect of the posterior cricoid cartilage below the level of the true vocal folds; Type III extends completely through the entire cricoid cartilage; Type IV extends to the intrathoracic trachea. Laryngeal cleft occurs due to abnormal embryogenesis, due to the absence of the interaretinoid muscle, the first type develops, the second type occurs due to incomplete develop-

ment of the posterior cricoid cartilage, the third and fourth types due to incomplete formation of the tracheoesophageal septum. Depending on the degree of cleft, the symptoms and the time of their manifestation may be different. In infants born with a low degree of cleft, the course may be asymptomatic at first. The main symptoms are respiratory distress, frequent coughing, recurrent croup or stridor, and recurrent respiratory tract infections. Deeper clefts of the larynx manifest more severely and quickly after birth and may present with severe respiratory distress immediately after birth. The most serious consequences of an undiagnosed laryngeal cleft are chronic aspiration and recurrent aspiration infections that can lead to lung damage.

Medical treatment is possible for type 1 laryngeal clefts, which includes primarily thickening of the fluid and food to prevent aspiration, as well as treatment of associated diseases that may lead to swallowing dysfunction or additional swelling (e.g. GERD or food allergies). Types 2, 3, and 4 clefts should be managed through surgical treatment. For type 1 and type 2 clefts, and with the rare exception of type 3, endoscopic cleft repair is the method of choice. Surgical treatment of clefts of types III and IV is mainly performed via the open approach [37].

Laryngeal cysts and Laryngocele

Congenital laryngeal cysts are a rare congenital anomaly occurring in 1.82 [38] to 3.49 [39] per 100,000 newborns or 2% of all patients with stridor [39] (congenital laryngeal saccular cysts represent 1.5% of all patients with stridor) [40]. There are several classifications of congenital laryngeal cysts, among which the classification of DeSanto [41], who identified saccular and ductal types of cysts, should be highlighted. Forte's classification, which was based on the differentiation into types as a basis for treatment approaches, defined two types depending on the size and embryologic tissue origin. Type I cysts consist only of endodermal elements are located entirely in the larynx, and can be removed completely with endoscopic surgery. Cysts of the second type have extralaryngeal extension, open surgical access is used for their removal, and depending on the presence of mesodermal tissues in addition to endodermal tissues, the author divided the type into additional subtypes 2a and 2b [42]. The most common symptoms in children with laryngeal cysts are stridor, feeding difficulties and failure to thrive, episodes of cyanosis. It should also be noted that in recent studies [43] it was reported that 2/3 of patients with vallecular cysts had coexisting laryngomalacia, and in a study by Yang Xiao et al. 35.7% of patients with congenital laryngeal saccular cysts were misdiagnosed as laryngomalacia [44]. Flexible fiberoptic laryngoscopy is used for initial diagnosis, endoscopic laryngotracheoscopy under general anesthesia is the gold standard, and CT scanning can also be useful for the evaluation of cyst extralaryngeal extension [44]. Treatment of congenital laryngeal cysts consists of surgical removal or marsupialization. In the case of ductal cysts and saccular cysts without extralaryngeal extension, it is recommended to perform surgery at the time of diagnostic laryngotracheoscopy [45].

Laryngocele is a very rare congenital anomaly of the larynx, which is an air-filled cystic dilated laryngeal saccule [46]. It is manifested by a stridor, in severe cases it can cause respiratory distress, and significant airway obstruction in the infant, and can be life-threatening [47]. A tracheostomy should be performed as an emergency treatment for a newborn with severe dyspnoea and laryngocele. Treatment of laryngocele is only surgical, marsupialisation of the cyst walls under endoscopic control is the optimal minimally invasive treatment.

Conclusions

Although stridor is usually a symptom of laryngomalacia, which in most cases has a benign course, this symptom is not specific only to laryngomalacia and can be a sign indicating the presence of another serious disease. Children with stridor require significant attention and appropriate diagnostics because stridor can be a manifestation of many different laryngeal pathologies in terms of symptoms, etiology, course, and consequences, which may require completely different approaches to the treatment of such patients. Stridor in children after birth should not be underestimated or ignored.

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References

1. Thompson DM. Abnormal sensorimotor integrative function of the larynx in congenital laryngomalacia: a new theory of etiology. Laryngoscope. 2007 Jun;117(6 Pt 2 Suppl 114):1-33. doi: 10.1097/MLG.0b013e31804a5750.
2. Daniel SJ. The upper airway: congenital malformations. Paediatr Respir Rev. 2006;7 Suppl 1:S260-3. doi: 10.1016/j.prrv.2006.04.227.
3. Richter GT, Thompson DM. The surgical management of laryngomalacia. Otolaryngol Clin North Am. 2008 Oct;41(5):837-64, vii. doi: 10.1016/j.otc.2008.04.011.
4. Kusak B, Cichocka-Jarosz E, Jedynak-Wasowicz U, Lis G. Types of laryngomalacia in children: interrelationship between clinical course and comorbid conditions. Eur Arch Otorhinolaryngol. 2017 Mar;274(3):1577-1583. doi: 10.1007/s00405-016-4334-5.
5. Munson PD, Saad AG, El-Jamal SM, Dai Y, Bower CM, Richter GT. Submucosal nerve hypertrophy in congenital laryngomalacia. Laryngoscope. 2011 Mar;121(3):627-9. doi: 10.1002/lary.21360.
6. Yeung JC, Balakrishnan K, Cheng ATL, Daniel SJ, Garabedian EN, Hart CK, Inglis AF Jr, Leboulanger N, Liming BJ, Moreddu E, Nicollas R, Russell JD, Rutter MJ, Sidell DR, Spratley J, Soma M, Thierry B, Thompson DM, Triglia JM, Watters K, Wyatt M, Zalzal GH, Zur KB, Rahbar R. International Pediatric Otolaryngology Group: Consensus guidelines on the diagnosis and management of type I laryngeal clefts. Int J Pediatr Otorhinolaryngol. 2017 Oct;101:51-56. doi: 10.1016/j.ijporl.2017.07.016.
7. Lesnik M, Thierry B, Blanchard M, Glynn F, Denoyelle F, Couloigner V, Garabedian N, Leboulanger N. Idiopathic bilateral vocal cord paralysis in infants: Case series and literature review. Laryngoscope. 2015 Jul;125(7):1724-8. doi: 10.1002/lary.25076.
8. Pfleger A, Eber E. Assessment and causes of stridor. Paediatr Respir Rev. 2016 Mar;18:64-72. doi: 10.1016/j.prrv.2015.10.003.
9. Jabbour J, Martin T, Beste D, Robey T. Pediatric vocal fold immobility: natural history and the need for long-term follow-up. JAMA Otolaryngol Head Neck Surg. 2014 May;140(5):428-33. doi: 10.1001/jamaoto.2014.81.
10. Jephson CG, Mills NA, Pitt MC, Beeson D, Aloysius A, Muntoni F, Robb SA, Bailey CM. Congenital stridor with feeding difficulty as a presenting symptom of Dok7 congenital myasthenic syndrome. Int J Pediatr Otorhinolaryngol. 2010 Sep;74(9):991-4. doi: 10.1016/j.ijporl.2010.05.022.
11. Arora N, Juneja R, Meher R, Bhargava EK. Bilateral Vocal Cord Palsy with Arnold Chiari Malformation: A Rare Case Series. J Clin Diagn Res. 2016 Sep;10(9):MR01-MR03. doi: 10.7860/JCDR/2016/20135.8443.
12. Harnisch W, Brosch S, Schmidt M, Hagen R. Breathing and voice quality after surgical treatment for bilateral vocal cord paralysis. Arch Otolaryngol Head Neck Surg. 2008 Mar;134(3):278-84. doi: 10.1001/archoto.2007.44.
13. King EF, Blumin JH. Vocal cord paralysis in children. Curr Opin Otolaryngol Head Neck Surg. 2009 Dec;17(6):483-7. doi: 10.1097/MOO.0b013e328331b77e.
14. Brigger MT, Hartnick CJ. Surgery for pediatric vocal cord paralysis: a meta-analysis. Otolaryngol Head Neck Surg. 2002 Apr;126(4):349-55. doi: 10.1067/mhn.2002.124185.
15. Hartnick CJ, Brigger MT, Willging JP, Cotton RT, Myer CM 3rd. Surgery for pediatric vocal cord paralysis: a retrospective review. Ann Otol Rhinol Laryngol. 2003 Jan;112(1):1-6. doi: 10.1177/000348940311200101.
16. Daya H, Hosni A, Bejar-Solar I, Evans JN, Bailey CM. Pediatric vocal fold paralysis: a long-term retrospective study. Arch Otolaryngol Head Neck Surg. 2000 Jan;126(1):21-5. doi: 10.1001/archotol.126.1.21.
17. Rutter MJ. Congenital laryngeal anomalies. Braz J Otorhinolaryngol. 2014 Nov-Dec;80(6):533-9. doi: 10.1016/j.bjorl.2014.08.001.
18. Sakakura K, Chikamatsu K, Toyoda M, Kaai M, Yasuoka Y, Furuya N. Congenital laryngeal anomalies presenting as chronic stridor: a retrospective study of 55 patients. Auris Nasus Larynx. 2008 Dec;35(4):527-33. doi: 10.1016/j.anl.2007.12.001.
19. Jefferson ND, Cohen AP, Rutter MJ. Subglottic stenosis. Semin Pediatr Surg. 2016 Jun;25(3):138-43. doi: 10.1053/j.sempedsurg.2016.02.006.
20. Marston AP, White DR. Subglottic Stenosis. Clin Perinatol. 2018 Dec;45(4):787-804. doi: 10.1016/j.clp.2018.07.013.
21. Smith ME, Elstad M. Mitomycin C and the endoscopic treatment of laryngotracheal stenosis: are two applications better than one? Laryngoscope. 2009 Feb;119(2):272-83. doi: 10.1002/lary.20056.
22. George M, Jaquet Y, Ikonomidis C, Monnier P. Management of severe pediatric subglottic stenosis with glottic involvement. J Thorac Cardiovasc Surg. 2010 Feb;139(2):411-7. doi: 10.1016/j.jtcvs.2009.05.010.
23. Milczuk HA, Smith JD, Everts EC. Congenital laryngeal webs: surgical management and clinical embryology. Int J Pediatr Otorhinolaryngol. 2000 Jan 30;52(1):1-9. doi: 10.1016/s0165-5876(99)00284-0.
24. de Trey LA, Lambercy K, Monnier P, Sandu K. Management of severe congenital laryngeal webs - a 12 year review. Int J Pediatr Otorhinolaryngol. 2016 Jul;86:82-6. doi: 10.1016/j.ijporl.2016.04.006.
25. Cohen SR. Congenital glottic webs in children. A retrospective review of 51 patients. Ann Otol Rhinol Laryngol Suppl. 1985 Nov-Dec;121:2-16. PMID: 3935032.
26. Lim FY, Crombleholme TM, Hedrick HL, Flake AW, Johnson MP, Howell LJ, Adzick NS. Congenital high airway obstruction syndrome: natural history and management. J Pediatr Surg. 2003 Jun;38(6):940-5. doi: 10.1016/s0022-3468(03)00128-3.

27. Wyatt ME, Hartley BE. Laryngotracheal reconstruction in congenital laryngeal webs and atresias. Otolaryngol Head Neck Surg. 2005 Feb;132(2):232-8. doi: 10.1016/j.otohns.2004.09.032.
28. Lawlor CM, Dombrowski ND, Nuss RC, Rahbar R, Choi SS. Laryngeal Web in the Pediatric Population: Evaluation and Management. Otolaryngol Head Neck Surg. 2020 Feb;162(2):234-240. doi: 10.1177/0194599819893985.
29. Abe Y, Hirade T, Koike D, Matama C, Kato F. Laryngeal web with 22q11.2 deletion syndrome. Int J Pediatr Adolesc Med. 2022 Sep;9(3):182-184. doi: 10.1016/j.ijpam.2022.02.001.
30. Monnier P. Subglottic Haemangioma (SGH). In: Pediatric Airway Surgery. Berlin, Heidelberg: Springer Berlin Heidelberg; 2011. p. 133–9.
31. Buckmiller LM, Munson PD, Dyamenahalli U, Dai Y, Richter GT. Propranolol for infantile hemangiomas: early experience at a tertiary vascular anomalies center. Laryngoscope. 2010 Apr;120(4):676-81. doi: 10.1002/lary.20807.
32. Bajaj Y, Kapoor K, Ifeacho S, Jephson CG, Albert DM, Harper JI, Hartley BE. Great Ormond Street Hospital treatment guidelines for use of propranolol in infantile isolated subglottic haemangioma. J Laryngol Otol. 2013 Mar;127(3):295-8. doi: 10.1017/S0022215112003192.
33. Munden A, Butschek R, Tom WL, Marshall JS, Poeltler DM, Krohne SE, Alió AB, Ritter M, Friedlander DF, Catanzarite V, Mendoza A, Smith L, Friedlander M, Friedlander SF. Prospective study of infantile haemangiomas: incidence, clinical characteristics and association with placental anomalies. Br J Dermatol. 2014 Apr;170(4):907-13. doi: 10.1111/bjd.12804.
34. Ida JB, Thompson DM. Pediatric stridor. Otolaryngol Clin North Am. 2014 Oct;47(5):795-819. doi: 10.1016/j.otc.2014.06.005.
35. Strychowsky JE, Rahbar R. Laryngotracheoesophageal clefts. Semin Pediatr Surg. 2016 Jun;25(3):128-31. doi: 10.1053/j.sempedsurg.2016.02.005.
36. Benjamin B, Inglis A. Minor Congenital Laryngeal Clefts: Diagnosis and Classification. Annals of Otology, Rhinology & Laryngology. 1989;98(6):417-420. doi:10.1177/000348948909800603.
37. Johnston DR, Watters K, Ferrari LR, Rahbar R. Laryngeal cleft: evaluation and management. Int J Pediatr Otorhinolaryngol. 2014 Jun;78(6):905-11. doi: 10.1016/j.ijporl.2014.03.015.
38. Pak MW, Woo JK, van Hasselt CA. Congenital laryngeal cysts: current approach to management. J Laryngol Otol. 1996 Sep;110(9):854-6. doi: 10.1017/s0022215100135157.
39. Birch DA. Laryngeal Stridor in Infants and Children. J Laryngol Otol. 1961 Sep 29;75(9):833–40.
40. Monnier P. Ductal Cysts, Saccular Cysts and Laryngoceles. In: Pediatric Airway Surgery. Berlin, Heidelberg: Springer Berlin Heidelberg; 2011. p. 141–5.
41. DeSanto LW, Devine KD, Weiland LH. Cysts of the larynx--classification. Laryngoscope. 1970 Jan;80(1):145-76. doi: 10.1288/00005537-197001000-00013.
42. Forte V, Fuoco G, James A. A new classification system for congenital laryngeal cysts. Laryngoscope. 2004 Jun;114(6):1123-7. doi: 10.1097/00005537-200406000-00031.
43. Prowse S, Knight L. Congenital cysts of the infant larynx. Int J Pediatr Otorhinolaryngol. 2012 May;76(5):708-11. doi: 10.1016/j.ijporl.2012.02.025.
44. Xiao Y, Wang J, Ma L, Han D. The clinical characteristics of congenital laryngeal saccular cysts. Acta Otolaryngol. 2016;136(2):168-71. doi: 10.3109/00016489.2015.1100327.
45. Hsieh WS, Yang PH, Wong KS, Li HY, Wang EC, Yeh TF. Vallecular cyst: an uncommon cause of stridor in newborn infants. Eur J Pediatr. 2000 Jan-Feb;159(1-2):79-81. doi: 10.1007/pl00013809.
46. Erdogmus B, Yazici B, Ozturk O, Ataoglu S, Yazici S. Laryngocele in association with ankylosing spondylitis. Wien Klin Wochenschr. 2005 Oct;117(19-20):718-20. doi: 10.1007/s00508-005-0460-6.
47. Ahmed H, Ndiaye C, Barry MW, Thiongane A, Mbaye A, Zemene Y, Ndiaye IC. A Rare Cause of Upper Airway Obstruction in a Child. Case Rep Otolaryngol. 2017;2017:2017265. doi: 10.1155/2017/2017265.

REVIEW PAPER

JMS Journal of Medical Science

The medicinal properties of clove with special focus on antimicrobial effect: a systematic review

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ABSTRACT

Clove, the dried flower bud of the *Syzygium aromaticum* tree, has been used for thousands of years in traditional medicine for its medicinal properties. One of the most well-known properties of clove is its antimicrobial activity attributed to a compound called eugenol. It has been found to be effective against a wide range of microorganisms, including bacteria, viruses, fungi, and parasites. In addition to its antimicrobial activity, clove has also been found to have anti-inflammatory, antioxidant, and pain-relieving properties. These properties make it an ideal natural remedy for a wide range of conditions, including toothaches, sore throat, and skin infections. With this background a systematic review was conducted wherein we explored the articles relevant to the research questions through databases like PubMed, Scopus, Web of Science, etc. Out of 150 articles explored, 30 were selected through online and snowballing literature searches. Out of these, 19 were original research articles, 8 systematic reviews, and three books/online resources. The selected articles were further assessed on quality reporting. In the current scenario of increasing prevalence of bacterial infections and emergence of multidrug resistant strains it is essential to explore alternative options like herbal antimicrobials to combat this problem.

Introduction

Clove, the dried flower bud of the *Syzygium aromaticum* tree, has been used for thousands of years in traditional medicine for its medicinal properties. One of the most well-known properties of clove is its antimicrobial activity.

Clove contains a compound called eugenol, which is responsible for its antimicrobial activity. Eugenol has been found to be effective against

a wide range of microorganisms, including bacteria, viruses, fungi, and parasites. In particular, it has been found to be effective against some of the most common bacterial and fungal pathogens, such as *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* [1,2].

In addition to its antimicrobial activity, clove has also been found to have anti-inflammatory, antioxidant, and pain-relieving properties. These properties make it an ideal natural remedy for a wide range of conditions, including toothaches, sore throat, and skin infections [3,4].

Clove oil is the most commonly used form of clove in traditional medicine, and it is used as a natural remedy for toothaches, sore throat, and skin infections. The oil is also used as a natural remedy for headaches and as an insect repellent [5].

With this background a systematic review was conducted wherein we explored the articles relevant to the research questions through databases like PubMed, Scopus, Web of Science, etc. Out of 150 articles explored, 30 were selected through online and snowballing literature searches. Out of these, 19 were original research articles, 8 systematic reviews, and 3 books/online resources. The selected articles were further assessed on quality reporting.

Table 1. Taxonomy and Scientific Clssification of Clove.

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Myrtales
Family	Myrtaceae
Genus	Syzygium
Species	aromaticum

Clove oil can also be used as a natural preservative for food, as it has been found to be effective in preventing the growth of bacteria and fungi. The oil can be added to food products in small amounts to help preserve their freshness.

Clove oil is also commonly used in aromatherapy, as its strong, spicy aroma is believed to have a soothing and relaxing effect on the mind and body [6].

Syzygium aromaticum, commonly known as clove, is a small, evergreen tree native to tropical regions. It is also referred to as *Eugenia aromaticum* or *Eugenia caryophyllata*. The plant produces highly aromatic, unopened flower buds that are harvested and dried for use as a spice. The spice is widely recognized by the same name as the tree and is often used in its plural form as "cloves" [7].

Cloves are highly valued for their culinary, medicinal, and commercial uses. As a culinary spice, cloves are widely used for their strong aroma and pungent taste, which adds a unique flavor to various dishes from different cuisines around the world. The essential oil extracted from the buds is highly valued for its flavoring and perfuming properties, as well as its medicinal properties, such as antiseptic, analgesic, and anesthetic effects [5].

The origins of clove can be traced back to the Spice Islands (Maluku Islands), where it played a central role in the early spice trade. Cloves were highly prized and were considered a valuable commodity that was sought after by traders from all over the world. Even today, clove remains an important spice and is used in various applications, including cooking, perfumery, and medicine. Its unique flavor and medicinal properties make it a popular ingredient in a variety of traditional and modern preparations [5].

The Myrtle family Myrtaceae, to which the clove tree belongs, is a group of dicotyledon plants categorized under the order Myrtales. This family is comprised of 130–150 genera and has over 3000 species, including popular plants like guava, myrtle, allspice, eucalyptus, and feijoa. The species in this family are typically woody, with essential oils, and have flower parts in multiples of four or five. Although the flowers usually have a base number of five petals, some genera have very minute or even absent petals. The stamens are usually numerous, brightly coloured, and easily noticeable. The leaves of the Myrtaceae family are simple, alternate or opposite, evergreen, and have an entire margin. A notable feature of this family is that the phloem can be found on both sides of the xylem, not just outside, as is common in most other plants [8].

The clove tree, *Syzygium aromaticum*, is a conical evergreen that grows to a height ranging from ten to 20 meters, having large oval leaves and crimson flowers in numerous groups of terminal clusters. The flower buds are at first pale in colour and gradually become green, after which they develop into a reddish brown or bright

red when they are ready for collecting. Cloves are harvested when 1.5 to two centimetres (cm) long and consist of a long calyx, terminating in four spreading sepals and four unopened petals that form a small ball in the centre. The flower buds are strongly aromatic and impart a flavour that can be described as hot and pungent [9].

The term "clove" derives from the French word clou, meaning nail, as the buds resemble small, irregular nails in shape. According to the Food and Agriculture Organization (FAO), Indonesia produced almost 80 percent of the world's clove output in 2005, followed at a distance by Madagascar and Tanzania. Cloves are also cultivated in Pakistan, India, Sri Lanka, Mauritius, and the West Indies. Cloves are one of the world's most essential, popular, and useful plants, It is commonly used as a culinary spice, adding a strong aroma and pungent flavor to a variety of dishes. The essential oil extracted from cloves is also highly prized for its various uses in flavourings, perfumes, and medicinal properties, including antiseptic, analgesic, and anaesthetic properties [10].

Chemical compounds isolated from clove

A diverse array of phenolic compounds is found in clove, one of the major vegetal sources of these compounds. Clove contains various phenolic compounds such as flavonoids, hydroxybenzoic acids, hydroxycinnamic acids, and hydroxyphenyl propens, with eugenol being the predominant bioactive compound present in fresh plant material. Gallic acid is also present in high concentrations, with 783.50 mg/100 g fresh weight, along with other gallic acid derivatives such as hydrolyzable tannins, which are present in concentrations of 2,375.8 mg/100 g. Additionally, the clove plant contains other phenolic acids like caffeic, ferulic, ellagic, and salicylic acids, as well as flavonoids like kaempferol and quercetin and their glycosylated derivatives, albeit in lower concentrations [11].

The clove flower buds can contain up to 18% essential oil, with eugenol accounting for approximately 89% of the oil content, while eugenol acetate and β-caryophyllene make up 5% to 15%. α-humulene is another important compound present in the essential oil of clove, with concentrations of up to 2.1%. β-pinene, benzaldehyde, farnesol, limonene, 2-heptanone, and ethyl hexanoate are some other compounds of volatile nature that are present in clove oil [4].

Biologically active compounds

Eugenol is the primary compound responsible for the unique and strong aroma of cloves. It is present in the essential oil obtained from cloves and constitutes a significant portion ranging from 72 to 90 percent of the oil's composition. Eugenol is widely known for its potent antiseptic and anaesthetic properties and is commonly used in mouthwashes and germicides [3].

Apart from eugenol, there are several other active compounds found in cloves that contribute to their various medicinal properties. Acetyl eugenol, beta-caryophyllene, and vanillin are among the other essential oils present in cloves, while crategolic acid, tannins, and gallotannic acid are non-volatile compounds with pharmacological activity [12].

Methyl salicylate, one of the major constituents of wintergreen oil, is also present in cloves and acts as a painkiller.Flavonoids such as eugenin, kaempferol, rhamnetin, and eugenitin also add up to its medicinal benefits. Oleanolic acid, stigmasterol, and campesterol are some triterpenoids that have been identified in clove extracts [13].

The combination of these active compounds is responsible for the numerous therapeutic properties of cloves, including their analgesic, antiseptic, anti-inflammatory, antioxidant, and anticancer effects. The use of cloves as a traditional medicine dates back centuries and continues to be an important part of many natural medicine practices around the world [14].

Medicinal properties of clove

Antibacterial and antifungal properties

The antimicrobial properties of clove have been extensively studied against various bacteria and fungi. Sofia et al. conducted a study to assess the antimicrobial effects of several Indian spice plants, including cinnamon, garlic, ginger, mint, mustard, and clove, against food-borne patho-

gens such as *Escherichia coli (E. coli), Staphylococcus aureus*, and *Bacillus cereus*. The results showed that only clove's aqueous extract, at a concentration of 3%, demonstrated complete bactericidal activity against all the tested pathogens. At a lower concentration of 1%, clove extract displayed significant inhibitory activity [15].

Another study by Dorman and Deans evaluated the antibacterial activity of black pepper, clove, geranium, nutmeg, oregano, and thyme against 25 strains of Gram-positive and Gram-negative bacteria. The oils with the broadest spectrum of activity were oregano, thyme, and clove, respectively [16].

Clove, oregano, bay, and thyme essential oils' antibacterial activity against *E. coli* O157:H7 was tested, with different levels of inhibition observed. Additionally, formulations containing eugenol and carvacrol encapsulated in a non-ionic surfactant were tested against *E. coli* O157:H7 and *Listeria monocytogenes*. The results demonstrated that eugenol could be used to inhibit the growth of these microorganisms on food-contact surfaces [17].

Rana et al. investigated the antifungal activity of clove oil against different strains and reported that it was most effective against Mucor sp., followed by *Microsporum gypseum, Fusarium moniliforme NCIM 1100, Trichophyton rubrum, Aspergillus sp.,* and *Fusarium oxysporum* MTCC 284. The antifungal activity of eugenol was demonstrated by the lysis of spores and micelles in the chromatographic analysis [18].

The efficacy of pure clove oil or clove oil mixed with rosemary oil was assessed against various bacteria, including *Staphylococcus epidermidis, Staphylococcus aureus, Bacillus subtilis, E. coli, Proteus vulgaris,* and *Pseudomonas aeruginosa*. The results indicated minimum inhibitory concentrations ranging from 0.062% to 0.500% (v/v), highlighting the potential use of clove oil as an anti-infectious agent or food preservative [19].

Eugenol and carvacrol's anticandidal activity was evaluated in a vaginal candidiasis model, and the results indicated that these compounds could be promising antifungal agents for the treatment and prophylaxis of vaginal candidiasis [20].

Furthermore, a study demonstrated that eugenol and cinnamaldehyde inhibited the growth of 31 strains of *Helicobacter pylori* at 2 µg/mL after 9 and 12 hours of incubation, respectively, without developing resistance. The activity and stability of these compounds were also examined at low pH values since *Helicobacter pylori* resides in the stomach [21].

Solid lipid nanoparticles containing eugenol were developed using stearic acid, caprylic triglyceride, and Poloxamer 188 at various concentrations by a modified hot homogenisation ultrasonication method. The particles were characterised by their particle size, polydispersity index, morphology, zeta potential, crystalline state, and encapsulation efficiency [22].

Anti-oxidant effects

Based on the database created by the United States Department of Agriculture, Perez-Jimenez et al. classified the 100 richest dietary sources of polyphenols and concluded that spice plants have the highest polyphenol content, followed by fruits, seeds, and vegetables [23].

Among all spices, cloves contain the highest amounts of polyphenol and antioxidant compounds. Shan et al. identified and quantified the main phenolic compounds in 26 spices by high-performance liquid chromatography and in vitro antioxidant activity analysis. Results showed that clove had the highest antioxidant activity and polyphenol content [24].

The antioxidant activity of aqueous extracts of clove was tested by different in vitro methods, and clove and other plants proved to have enormous potential as food preservatives. The powerful antioxidant activity of ethanol and aqueous extracts of clove and lavender may be attributed to their strong hydrogen-donating ability, metal-chelating ability, and scavenging of free radicals, hydrogen peroxide, and superoxide [25].

A study exposed male rats to hepato-toxic and nephrotoxic stress showed higher antioxidant effect of eugenol when compared to *S. aromaticum* oil and *Nigella sativa* oil [4].

Oxidative stress can also impact brain cells leading to cognitive decline and memory deficits. A study on mice's brain demonstrated the positive effects of clove oil in reducing oxidative stress. Implications of clove oil in reverting memory and learning deficits in some cases need to be studied more [26].

Anti-viral properties

Researchers conducted a study to test the antiviral activity of eugeniin, a compound derived from

S. aromaticum and *Geum japonicum*, against various herpes virus strains. The results showed that eugeniin was effective at a concentration of 5 µg/mL, and it was found that one of the primary targets of eugeniin was the inhibition of viral DNA synthesis through the inhibition of viral DNA polymerase. This finding suggests that eugeniin has the potential to be developed as an antiviral drug [4].

In another study, researchers examined the antiviral activity of aqueous extracts from several plants, including *S. aromaticum, Geum japonicum, Rhus javanica*, and *Terminalia chebula*, against *Herpes simplex* virus type 1 (HSV-1) when combined with acyclovir. The results showed that the combination of these extracts with acyclovir exhibited strong synergistic activity against HSV-1, with the strongest activity observed in the brain compared to the skin. Furthermore, it was demonstrated that these combinations were non-toxic to mice. These findings suggest that a combination of these plant extracts and acyclovir may be an effective treatment option for HSV-1 infections [27].

Analgesic action

Since the 13th century, clove has been employed as an analgesic for relieving toothaches, joint pain, and spasms. The main compound responsible for this activity is eugenol. It has been observed that the mechanism of action involves the activation of calcium and chloride channels in ganglion cells. This activation, in turn, leads to a reduction in pain. The analgesic effect of clove can also be attributed to the voltage-dependent effects of eugenol in sodium and calcium channels and in receptors expressed in the trigeminal ganglion. The activation of these channels and receptors contributes to the analgesic effect of clove. Another study has shown that the analgesic effect of clove is due to the action of eugenol as a capsaicin agonist. Eugenol has also been reported to have significant peripheral antinociceptive activity [28].

Studies have also shown the benefits of cloves in reducing metastasis of some cancers when given along with the chosen therapy. It is not proven to cure cancers individually but can be beneficial during recovery [29].

Toxicity and pharmacokinetics

According to scientific research, the consumption of clove essential oil in concentrations lower than 1,500 mg/kg is considered safe. Moreover, the World Health Organization (WHO) has established that the acceptable daily intake of clove for humans is 2.5 mg/kg of body weight. However, the toxicity of clove oil was tested in two species of aquarium fish, namely *Danio rerio* and *Poecilia reticulata*. The results showed that the medium lethal concentrations (LD50) at 96 hours were (18.2 ± 5.52) mg/mL and (21.7 ± 0.8) mg/mL, respectively [4].

When eugenol, the main component of clove essential oil, is administered orally, it is quickly absorbed and reaches the plasma and blood in a short period. The mean half-lives for plasma and blood are approximately 14.0 hours and 18.3 hours, respectively. It has been hypothesized that a cumulative effect may occur, which could be associated with the relief of neuropathic pain after repeated daily administrations [30].

Conclusion

Clove is a powerful natural remedy that has been used for centuries for its medicinal properties. Its antimicrobial activity is one of the most well-known properties of clove and it is effective against a wide range of microorganisms. Clove has also been found to have anti-inflammatory, antioxidant, and pain-relieving properties, making it a versatile natural remedy. Clove oil is the most commonly used form of clove in traditional medicine, it is widely used in dental care, aromatherapy, and food preservation. The potential of plant-based antimicrobials like cloves, neem, and turmeric may help devise an effective strategy to reduce antibiotic misuse and break the resistance among microbes.

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Conflict of interest statement
The authors declare no conflict of interest.

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All authors contributed significantly in the process and approved to the final version of manuscript.

References

1. Nuñez L, Aquino MD. Microbicide activity of clove essential oil (Eugenia caryophyllata). Braz J Microbiol. 2012 Oct;43(4):1255-60. doi: 10.1590/S1517-83822012000400003.
2. Jingwen B, Jianqiang L, Zhiyuan C, Xuedong B, Zhenyuan Y, Zitong W, et al. Antibacterial activity and mechanism of clove essential oil against food borne pathogens. LWT 2023;173:114249. doi: 10.1016/j.lwt.2022.114249.
3. Ulanowska M, Olas B. Biological Properties and Prospects for the Application of Eugenol-A Review. Int J Mol Sci. 2021 Apr 1;22(7):3671. doi: 10.3390/ijms22073671.
4. Cortés-Rojas DF, de Souza CR, Oliveira WP. Clove (Syzygium aromaticum): a precious spice. Asian Pac J Trop Biomed. 2014 Feb;4(2):90-6. doi: 10.1016/S2221-1691(14)60215-X.
5. Pandey VK, Srivastava S, Ashish, Dash KK, Singh R, Dar AH, Singh T, Farooqui A, Shaikh AM, Kovacs B. Bioactive properties of clove (Syzygium aromaticum) essential oil nanoemulsion: A comprehensive review. Heliyon. 2023 Nov 30;10(1):e22437. doi: 10.1016/j.heliyon.2023.e22437.
6. Vora LK, Gholap AD, Hatvate NT, Naren P, Khan S, Chavda VP, Balar PC, Gandhi J, Khatri DK. Essential oils for clinical aromatherapy: A comprehensive review. J Ethnopharmacol. 2024 Aug 10;330:118180. doi: 10.1016/j.jep.2024.118180.
7. Suppakul, P. Cinnamaldehyde and Eugenol: Use in Antimicrobial Packaging. Antimicrobial Food Packaging. 2016:479-490. doi: 10.1016/B978-0-12-800723-5.00039-5.
8. Kholiya S, Pandey H, Padalia RC, Tiwari A, Prakash O. Phytochemical analysis, post-harvest shade drying, and biological activities of Melaleuca linariifolia Sm. from foothills of Kumaun Himalayas, India. Biochemical Systematics and Ecology. 2024;114:104824. doi: 10.1016/j.bse.2024.104824.
9. Clove. (2023, January 14). New World Encyclopedia. Retrieved 00:36, October 21, 2024 from https://www.newworldencyclopedia.org/p/index.php?title=Clove&oldid=1095559.
10. Bruneton, J. (1995) Pharmacognosy, Phytochemistry and Medicinal Plants, English Translation by Hatton, C. K., Lavoisier Publishing, Paris, 265.
11. Guldiken B, Ozkan G, Catalkaya G, Ceylan FD, Ekin Yalcinkaya I, Capanoglu E. Phytochemicals of herbs and spices: Health versus toxicological effects. Food Chem Toxicol. 2018 Sep;119:37-49. doi: 10.1016/j.fct.2018.05.050.
12. Arshad U, Muhammad A, Hassan A. Role of Clove in Human Medical History. SAR Journal of Anatomy and Physiology 2023;4:10-19. doi: 10.36346/sarjap.2023.v04i02.001.
13. Liu H, Schmitz JC, Wei J, Cao S, Beumer JH, Strychor S, Cheng L, Liu M, Wang C, Wu N, Zhao X, Zhang Y, Liao J, Chu E, Lin X. Clove extract inhibits tumor growth and promotes cell cycle arrest and apoptosis. Oncol Res. 2014;21(5):247-59. doi: 10.3727/096504014X13946388748910.
14. Mekky AE, Emam AE, Selim MN. Antibacterial and antineoplastic MCF-7 and HePG-2 characteristics of the methanolic (80%)clove (Syzygium aromaticum L.) extract. Biomass Conv Bioref. 2024;14:16787-98. doi: 10.1007/s13399-023-03862-1.
15. Sofia PK, Prasad R, Vijay VK, Srivastava AK. Evaluation of antibacterial activity of Indian spices against common foodborne pathogens. Int J Food Sci Technol. 2007;42(8):910-15. doi: 10.1111/J.1365-2621.2006.01308.X.
16. Dorman HJ, Deans SG. Antimicrobial agents from plants:antibacterial activity of plant volatile oils. J Appl Microbiol. 2000;88(2):308-16. doi: 10.1046/j.1365-2672.2000.00969.x.
17. Pérez-Conesa D, McLandsborough L, Weiss J. Inhibition and inactivation of Listeria monocytogenes and Escherichia coli O157:H7 colony biofilms by micellar-encapsulated eugenol and carvacrol. J Food Prot. 2006 Dec;69(12):2947-54. doi: 10.4315/0362-028x-69.12.2947.
18. Rana IS, Rana AS, Rajak RC. Evaluation of antifungal activity in essential oil of the Syzygium aromaticum (L.) by extraction, purification and analysis of its main component eugenol. Braz J Microbiol. 2011 Oct;42(4):1269-77. doi: 10.1590/S1517-83822011000400004.
19. Fu Y, Zu Y, Chen L, Shi X, Wang Z, Sun S, Efferth T. Antimicrobial activity of clove and rosemary essential oils alone and in combination. Phytother Res. 2007 Oct;21(10):989-94. doi: 10.1002/ptr.2179.
20. Chami F, Chami N, Bennis S, Trouillas J, Remmal A. Evaluation of carvacrol and eugenol as prophylaxis and treatment of vaginal candidiasis in an immunosuppressed rat model. J Antimicrob Chemother. 2004 Nov;54(5):909-14. doi: 10.1093/jac/dkh436.
21. Ali SM, Khan AA, Ahmed I, Musaddiq M, Ahmed KS, Polasa H, Rao LV, Habibullah CM, Sechi LA, Ahmed N. Antimicrobial activities of Eugenol and Cinnamaldehyde against the human gastric pathogen Helicobacter pylori. Ann Clin Microbiol Antimicrob. 2005 Dec 21;4:20. doi: 10.1186/1476-0711-4-20.
22. Garg A, Singh S. Enhancement in antifungal activity of eugenol in immunosuppressed rats through lipid nanocarriers. Colloids Surf B Biointerfaces. 2011 Oct 15;87(2):280-8. doi: 10.1016/j.colsurfb.2011.05.030.
23. Pérez-Jiménez J, Neveu V, Vos F, Scalbert A. Identification of the 100 richest dietary sources of polyphenols: an application of the Phenol-Explorer database. Eur J Clin Nutr. 2010 Nov;64 Suppl 3:S112-20. doi: 10.1038/ejcn.2010.221.
24. Shan B, Cai YZ, Sun M, Corke H. Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. J Agric Food Chem. 2005 Oct 5;53(20):7749-59. doi: 10.1021/jf051513y.
25. Gulcin I, Elmastas M, Aboul-Enein HY. Antioxidant activity of clove oil-A powerful antioxidant source. Arab J Chem. 2012;5(4):489-99. doi: 10.1016/j.arabjc.2010.09.016
26. Halder S, Mehta AK, Kar R, Mustafa M, Mediratta PK, Sharma KK. Clove oil reverses learning and memory deficits in scopolamine-treated mice. Planta Med. 2011 May;77(8):830-4. doi: 10.1055/s-0030-1250605.

27. Yukawa TA, Kurokawa M, Sato H, Yoshida Y, Kageyama S, Hasegawa T, Namba T, Imakita M, Hozumi T, Shiraki K. Prophylactic treatment of cytomegalovirus infection with traditional herbs. Antiviral Res. 1996 Oct;32(2):63-70. doi: 10.1016/0166-3542(95)00978-7.
28. Daniel AN, Sartoretto SM, Schmidt G, Caparroz-Assef SM, Bersani-Amado CA, Cuman RKN. Anti-inflammatory and antinociceptive activities A of eugenol essential oil in experimental animal models. Rev bras farmacogn. 2009;19(1b):212–17. doi: 10.1590/S0102-695X2009000200006.
29. Nirmala MJ, Durai L, Gopakumar V, Nagarajan R. Anticancer and antibacterial effects of a clove bud essential oil-based nanoscale emulsion system. Int J Nanomedicine. 2019;14:6439-6450. doi: 10.2147/IJN.S211047.
30. Haro-Gonzalez JN, Castillo-Herrera GA, Martínez-Velazquez M, Espinosa-Andrews H. Clove Essential Oil (Syzygium aromaticum L.Myrtaceae):Extraction, Chemical Composition, Food Applications, and Essential Bioactivity for Human Health. Molecules. 2021;26(21):6387. doi: 10.3390/molecules26216387.

REVIEW PAPER

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Efficacy and safety of intravenous iron therapy in heart failure patients with iron deficiency: a systematic review and meta-analysis of randomized controlled trials

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ABSTRACT

Introduction. Heart failure is a diverse life-threatening condition with complex biology and demanding therapeutic goals. Even when anemic patients are excluded, up to 59% of heart failure patients have low ferritin levels, making them especially vulnerable to iron deficiency. We aim to explore the benefits and safety of intravenous iron therapy among patients with heart failure and iron deficiency.

Material and methods. We have searched the literature on PubMed (MEDLINE), Scopus, Cochrane Central Register of Controlled Trials (CENTRAL), Web of Science (WoS), and EMBASE until 31st August, 2023. We

used RevMan V. 5.4 to pool dichotomous data using a risk ratio (RR) with a 95% confidence interval (CI). This review has been registered and published in PROSPERO (CRD42023471419)
Results. Fourteen randomized controlled trials with 6,626 patients were included. The intravenous iron group was favored over the control group in reducing hospital admissions for heart failure (first event) (RR= 0.83, 95% CI 0.71 to 0.97; p = 0.02) and (total events) (RR= 0.81, 95% CI 0.74 to 0.89; p < 0.0001). Also, the iron group had a 21% lower risk in terms of cardiovascular death and hospital admission for heart failure (number of events, rate per 100 patients in a year) (RR= 0.79, 95% CI 0.74 to 0.85; p < 0.00001). Concerning the adverse events, both ferric carboxymaltose and ferric derisomaltose showed a beneficial effect in reducing the cardiac disorder (RR= 0.81, 95% CI 0.76 to 0.87; p <0.0001), and (RR= 0.82, 95% CI 0.71 to 0.95; p = 0.009), respectively.
Conclusions. Intravenous iron infusion in patients with heart failure has a favorable safety profile. It reduces total hospitalizations for heart failure and cardiovascular mortality, with no effect on all-cause mortality, cardiovascular mortality alone, or first-time hospitalization for heart failure.

Introduction

Heart failure (HF) is a multifaceted, life-threatening syndrome with a complex pathophysiology and challenging management goals. HF is a clinical syndrome characterized by symptoms and/or signs resulting from structural and/or functional cardiac abnormalities. This condition is confirmed by elevated levels of natriuretic peptides and/or objective evidence of pulmonary or systemic congestion. [1]. In 2017, The Global Burden of Disease report stated that 64.3 million people live with HF worldwide [2]. According to more recent estimates, one to three percent of individuals in low-income nations are believed to have HF. However, this incidence is expected to rise due to advancements in both diagnosis and treatment options that extend the lives of HF patients. In contrast, the incidence of HF has steadily declined over the past few decades, with an estimated 1–20 cases per 1,000 individuals identified annually [3].

Even after excluding anemic individuals, up to 59% of HF patients exhibit low ferritin levels, rendering them particularly susceptible to iron deficiency [4]. While the precise etiology of iron deficiency remains elusive, it has been suggested that increased iron depletion (due to gastrointestinal bleeding), reduced iron intake, absorption, and systemic bioavailability may contribute to the development of the disease [4]. It is crucial to note that iron deficiency in HF patients can manifest as either absolute (total body iron is decreased) or functional (total body iron is normal or increased). In the latter form, iron becomes sequestered in storage tissues (such as the liver), transferring insufficient amounts to the myocardium to meet its needs [5].

The activation of the neuroendocrine system can downregulate the messenger ribonucleic acid (mRNA) expression of Transferrin Receptor 1, leading to increased secretion of aldosterone and norepinephrine. Consequently, this downregulation can hinder iron uptake by cardiomyocytes [6]. The insufficient supply of iron to the myocardium gives rise to a condition known as myocardial iron deficiency, characterized by poor mitochondrial structure and function, oxidative stress, and increased detrimental cardiac remodeling [7]. Regardless of whether the iron deficiency is absolute or functional, it is associated with a poor prognosis in HF and has been demonstrated to be a robust and independent predictor of mortality [8].

Therefore, studies have aimed to evaluate the effectiveness of intravenous (IV) iron therapy in improving the condition of HF patients with iron deficiency. Among these, a multicenter randomized controlled trial (RCT) known as the CONFIRM-HF study, published in 2015, demonstrated the superiority of IV ferric carboxymaltose over placebo in ameliorating several outcomes, including functional capacity, symptoms, quality of life, martial deficiency, and hospitalization risks [9]. Other RCTs, such as the AFFIRM-AHF study, found that IV ferric carboxymaltose effectively lowers the risk of HF hospitalization among stabilized patients with iron deficiency and left ventricular ejection fraction < 50% after discharge from acute episodes, later corroborating these findings [10].

However, some results were not as positive. For example, the recent HEART-FID trial found no statistically significant difference in a hierarchical endpoint including mortality, HF hospitalizations, and six-minute walk distance between ambulatory HF patients with reduced ejection fraction and iron deficiency who took either ferric carboxymaltose or placebo [11], narrowly missing its prespecified target despite the large sample size.

In light of the ongoing controversy and inconsistency in the existing literature, we undertook a comprehensive systematic review and meta-analysis to evaluate the entirety of data derived from RCTs concerning the efficacy and safety of intravenous iron therapy in patients with HF and iron deficiency. The findings from our study hold substantial therapeutic implications.

Methods.

Protocol Registration

This systematic review and meta-analysis adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [12] and the Cochrane Handbook for systematic reviews and meta-analyses [13]. The review was registered and published in PROSPERO on 10th November 2023 under the ID CRD42023471419.

Data Sources & Search Strategy.

We have searched the literature on PubMed (MEDLINE), Scopus, Cochrane Central Register of Controlled Trials (CENTRAL), Web of Science (WoS), and EMBASE until 31st August 2023. We adjusted the search terms and keywords for each database; the results are presented in (**Table S1**).

Eligibility Criteria and Study Selection.

We included studies that followed the following PICOS criteria:

- Population (patients with HF and iron deficiency, no age criterion);
- Intervention (IV iron);
- Comparison (placebo or standard care);
- Outcomes:
  - Primary outcomes are (cardiovascular mortality, all-cause mortality, Hospital admission for heart failure (first event), hospital admission for heart failure (total event), cardiovascular death and hospital admission for heart failure (number of events, rate per 100 patients in a year), hospital admission for heart failure (number of events, rate per 100 patients in a year).
  - Secondary outcomes included adverse events: cardiac disorder, gastrointestinal disorder, injection site condition, infection, nervous system disorder, respiratory, thoracic, or mediastinal disorder, vascular disorder, any adverse effect, any serious adverse event, any adverse event leading to withdrawal, abnormal lab test, vital signs, or physical finding. Studies included were parallel RCTs.

Papers that met any of the following criteria were excluded: (1) non-original studies (e.g., book chapters, reviews, comments, letters to the editor, guidelines); (2) any other study design except RCTs; (3) studies involving duplicate or overlapping datasets; (4) non-human and in vitro experiments; and (5) studies not reported in English.

Study Selection.

We utilized the Covidence web tool to conduct the review. After eliminating duplicates, all obtained records were independently assessed by four authors. During the initial eligibility criteria full-text screening, the full texts of the records were reviewed by four authors. Any disagreements were resolved through discussion and consensus with a senior author.

Data Extraction.

After acquiring the full texts of relevant publications, we conducted a pilot extraction to effectively organize the data extraction sheet. The Excel-based data extraction sheet is divided into three sections.

The first part encompasses the summary characteristics of the included studies, such as the name of the first author, year of publication, country, follow-up period, population, iron preparation, comparator, iron dosing strategy, definition of iron deficiency, inclusion criteria, and primary outcome.

The second part consists of baseline information about the participants, covering race, The New York Heart Association (NYHA) class, age, gender, N-terminal prohormone of brain natri-

uretic peptide (NT-proBNP), B-type natriuretic peptide (BNP), left ventricular (LV) ejection fraction, 6-minute walk test distance (6MWT), systolic and diastolic blood pressure, hemoglobin, serum ferritin, transferrin saturation, estimated glomerular filtration rate (eGFR), phosphorus, hospital admissions for heart failure, de novo (new) hospital admissions for heart failure, comorbidities (atrial fibrillation (AF), acute coronary syndrome (ACS), hypertension, diabetes, chronic kidney disease (CKD), anemia, dyslipidemia), and medications (implantable cardioverter–defibrillator (ICD), cardiac resynchronization therapy (CRT), angiotensin-converting enzyme (ACE) inhibitor, angiotensin II receptor blocker (ARB), sacubitril–valsartan, ACE inhibitor, ARB, beta-blocker, mineralocorticoid receptor antagonist (MRA), digoxin, sodium-glucose cotransporter-2 (SGLT2) inhibitor, loop diuretic, insulin, and any other glucose-lowering medication). Finally, the third part covers outcomes data. Four reviewers (A.R., O.A., A.A., and I.U.) were responsible for data extraction, and any discrepancies were resolved through discussion and agreement with a senior author.

Risk of Bias and Certainty of Evidence.

Four reviewers (A.R., O.A., A.A., and I.U.) independently assessed the quality of the studies using the Cochrane RoB2 method [14]. Any disagreements were resolved through discussion with a senior author. Simultaneously, two reviewers (M.A. and B.A.) employed the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) criteria [15, 16] to assess the certainty of evidence. A consensus was reached to resolve any disagreements.

Statistical Analysis.

The statistical analysis was performed using RevMan v5.3 software. For dichotomous outcomes, we calculated the risk ratio (RR), and for continuous outcomes, we computed the mean difference (MD), both presented with a 95% confidence interval (CI) under the fixed-effects model. In cases of significant heterogeneity, we applied the random-effects model. Heterogeneity was assessed using the Chi-square and I-square tests; the Chi-square test determined the presence of heterogeneity, and the I-square test gauged its extent. As per the Cochrane Handbook (chapter nine) [17], an I-square exceeding 50% signified significant heterogeneity, while a Chi-square test with an alpha level below 0.1 indicated considerable heterogeneity.

We performed a subgroup analysis based on (i) chronic or acute heart failure and (ii) type of iron preparation. Furthermore, trial sequential analysis was employed to validate desired or undesired intervention effects by analyzing data from ongoing trials. Sensitivity analysis was also conducted to assess the impact of alternative assumptions or analyses on the pre-specified research questions. In essence, sensitivity analysis aims to evaluate the validity and certainty of the primary methodological or analytic strategy. Finally, if at least 10 studies were reported in the outcome, the asymmetry analysis was performed to determine the publication bias by visual inspection of the funnel plot of the studies, and Egger's test confirmed the results [18]. A p-value ≤ 0.05 was considered statistically significant for all tests.

Results

Study selection

Our database search yielded 2740 studies. After duplicate removal, we screened the remaining 1225 Studies, and only 35 were eligible for full-text retrieval. Only 14 studies met our inclusion criteria and were included in our review [9–11, 19–29] (**Figure 1**).

Study characteristics

Our included studies reported the data of a total of 6,626 patients who were assigned to IV iron as the intervention group (3,408 patients) or the control group (3,218 patients). The mean age of the Intervention group was 68.4 ± 4.95, and 68.1 ± 5.89 for the control group. Nine studies were single-centered, while the rest were multicenter studies. The follow-up duration ranged from two weeks to 2.7 years. The included studies' summary and detailed patient baseline characteristics are described in (**Table 1** and **2**), respectively [9–11, 19–29].

Risk of bias

The risk of bias assessment for each outcome is depicted in **Figure 2**. Overall, most included stud-

ies exhibited a low risk of bias across all assessed domains. Notably, two studies raised some concerns regarding bias (Karla et al. 2022: the data leading to this result was not analyzed as per the pre-specified analysis plan; Ponikowski et al. 2015: there is no evidence that the result was unaffected by missing outcomes, and the missingness in the outcome could be dependent on its true value). A GRADE evidence profile outlines The certainty of evidence (**Table 3**).

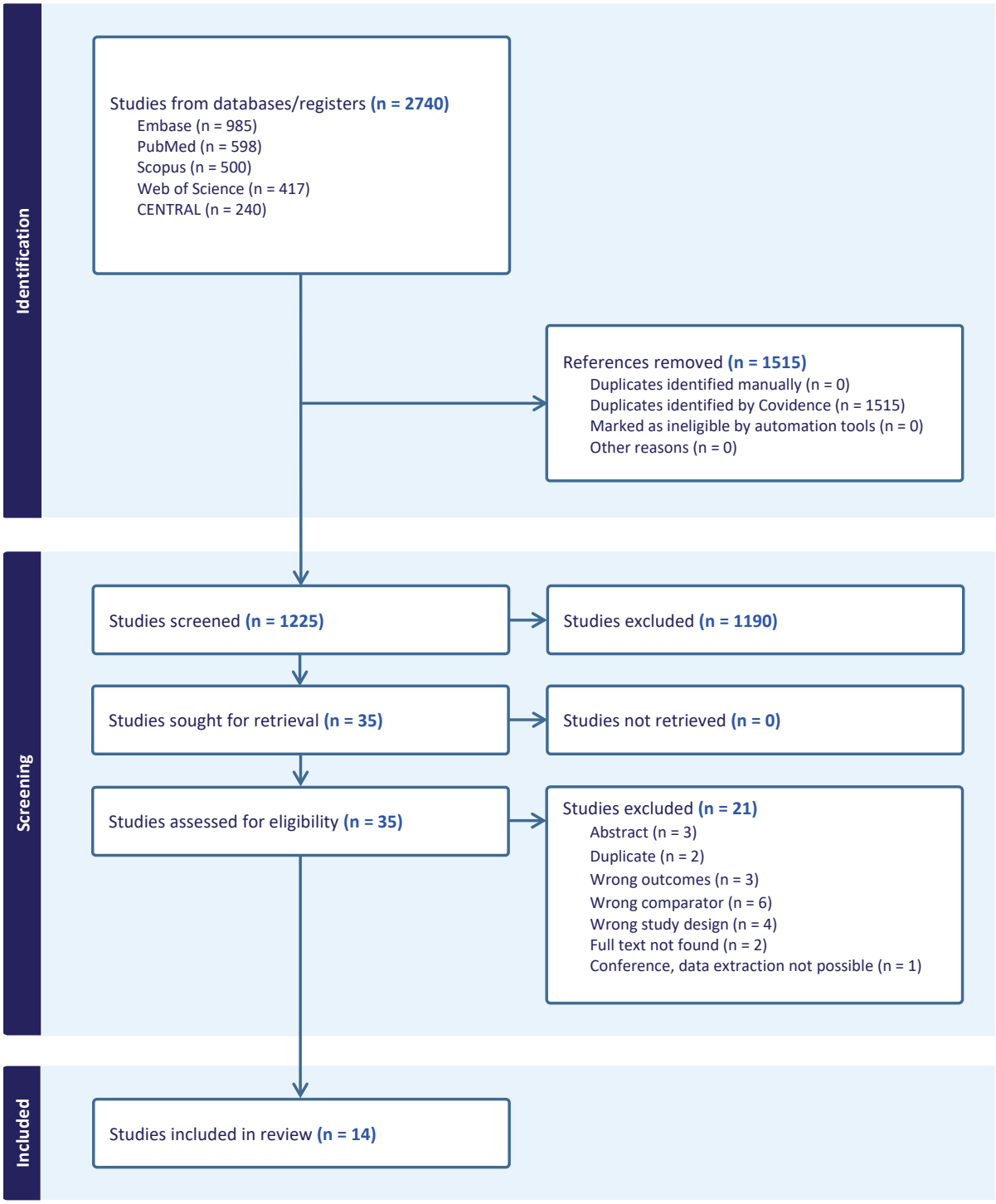

Figure 1. PRISMA chart showing the research strategy and inclusion and exclusion criteria.

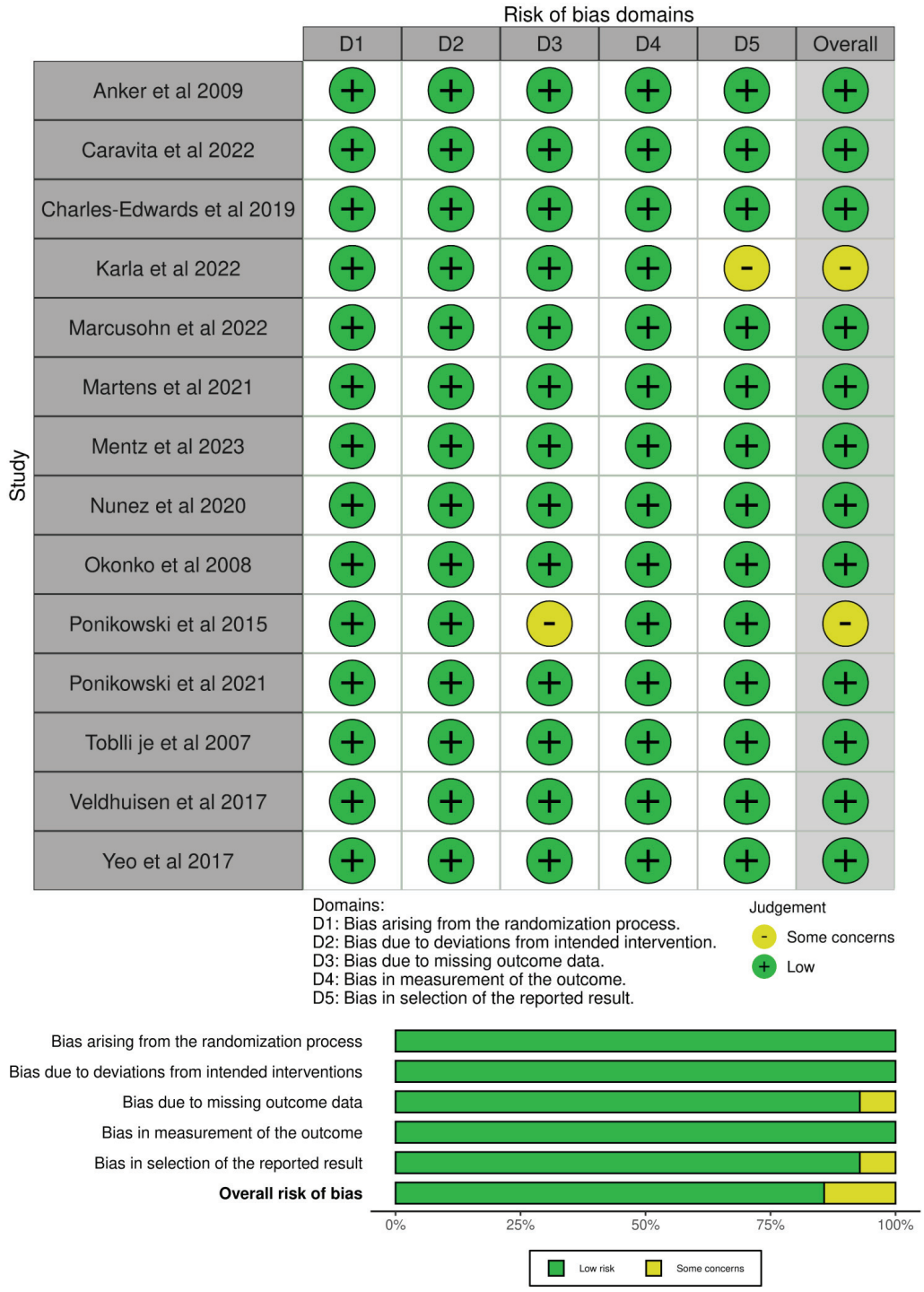

Figure 2. Risk of bias assessment is represented in traffic light and summary plots according to the Cochrane risk-of-bias tool, created using robvis.

Table 1. The summary of the included studies.

Ref.	Study	Year	Population	Comparison	Iron preparation	Iron dosing strategy	Country	Follow-up (week)	Iron deficiency	Definition of iron deficiency	Inclusion criteria	Primary outcome
[20]	Nunez et al	2020	Patients with stable chronic HF, left ventricular ejection fraction (LVEF) <50%, and ID (serum ferritin <100 lg/L	Placebo	Ferric Carboxymaltose	20-mL perfusion (equivalent to 1000 mg of iron)	Spain	30 days	Yes	ID (serum ferritin <100 lg/L [absolute ID] or 100–299 lg/L with transferrin saturation [TSAT] <20%	– Patients with ambulatory chronic heart failure Older than 18 years – Patients in NYHA class II-III on optimal background therapy – Elevated natriuretic peptides levels – Iron deficiency defined as: serum ferritin level <100 µg/L informed consent for participation in the study	Myocardial iron content
[21]	Okonko et al	2008	Anemic and Nonanemic Patients with Symptomatic Chronic Heart Failure and Iron Deficiency	Control	Iron sucrose	100mg at 0, 4, 8, 12 and 16 weeks	UK & Poland	18 weeks	Yes	Hb concentrations < 12.5 g/dl	– Age ≥ 21 years. – symptomatic CHF (New York Heart Association [NYHA] functional class II or III – Hb concentrations < 12.5 g/dl (anemic group) or 12.5 to 14.5 g/dl (nonanemic group) – Ferritin <100 µg/l or between 100 g/l and 300 µg/l – left ventricular ejection fraction ≤ 45%	Change in absolute pVO2 (ml/min) from baseline to week 18
[9]	Ponikowski et al	2015	Stable ambulatory HF patients	Placebo	Ferric Carboxymaltose	FCM doses were between 500 and 2000 mg iron FCM at each of Weeks 12, 24, and 36.	9 countries	24 weeks	Yes	Serum ferritin level ,100 ng/ mL, or between 100 and 300 ng/mL	– Iron deficient subjects with stable chronic heart failure – Reduced left ventricular ejection fraction Capable of completing 6-minute walk test. – At least 18 years of age.	Change in six-minute walk test
[10]	Ponikowski et al	2021	Patients With Acute Heart Failure	Placebo	Ferric Carboxymaltose	intravenously shortly before discharge and the second dose was administered at week 6 (visit three).	121 sites in Europe, South America, and Singapore	52 weeks	Yes	Iron deficient defined as serum ferritin <100 ng/mL or 100 ng/mL ≤ serum ferritin ≤299 ng/mL if TSAT <20%	– Hospitalised for an episode of acute heart failure (AHF) – Subject is iron deficient defined as serum ferritin <100 ng/mL. – Left ventricular ejection fraction <50% – Male or female aged ≥18 years old.	HF Hospitalizations and CV Death
[22]	Toblli je et al	2007	Anemic Patients with Chronic Heart Failure and Renal Insufficiency	Placebo	Iron sucrose	200 mg weekly	Argentina	6 months	Yes	Anemia with an iron deficit defined by Hb < 12.5 g/dl for men and < 11.5 g/dl for women.	– LV ejection fraction (EF) ≤ 35% – New York Heart Association (NYHA) functional class II to IV – anemia with an iron deficit defined by Hb < 12.5 g/dl	NT-pro-brain natriuretic peptide (NT-proBNP) and C-reactive protein (CRP) levels in a group of anemic patients with chronic heart failure (CHF) and chronic renal failure (CRF) receiving intravenous iron therapy.
[23]	Veldhuisen et al	2017	Patients with systolic HF (left ventricular ejection fraction ≤45%)	Placebo	Ferric Carboxymaltose	Infusions of 10 or 20 mL and Subjects will receive ferric carboxymaltose intravenously on Day 0, Week 6, and Week 12	Netherlands	24 weeks	Yes	Patients with Hb ≤14 g/dL	– Iron deficient subjects with stable chronic heart failure (CHF) – (NYHA II-III) on optimal background therapy for CHF – At least 18 years of age and	Effect of IV ferric carboxymaltose on exercise capacity, physical functioning and quality of life in patients with iron deficiency and chronic heart failure.
[24]	Yeo et al	2018	Asian patients with heart failure (HF)	Placebo	Ferric Carboxymaltose	1000mg intravenous Ferric Carboxymaltose	Singapore	12 weeks	Yes	Serum ferritin <300 ng/mL if transferrin saturation is <20%)	– Patients hospitalized for HF Capable of completing the 6MWT. – Screening TSAT <20%, Serum Ferritin <300 ng/mL and Hb≤14 g/dL – At least 21 years of age	Change in 6MWT distance over time

Ref.	Study	Year	Population	Comparison	Iron preparation	Iron dosing strategy	Country	Follow-up (week)	Iron deficiency	Definition of iron deficiency	Inclusion criteria	Primary outcome
[25]	Anker et al. (FAIR-HF)	2009	Patients who had chronic heart failure, left ventricular ejection fraction	Placebo	IV Ferric Carboxymaltose	4 ml weekly then every 4 weeks	Argentina, Czech Republic, Greece, Italy, Norway, Poland, Romania, Russia, Spain, Ukraine, and Germany	24 weeks	Yes	Serum ferritin level < 100 µg per liter when the transferrin saturation was <20%.	– patients who had chronic heart failure of New York Heart Association (NYHA) class II or III – Left ventricular ejection fraction of 40% or less a – hemoglobin level at the screening visit between 95 and 135 g per liter, and iron deficiency.	Patient Global Assessment and NYHA functional class, both at week 24.
[26]	Caravita et al.	2022	Patients with heart failure, reduced left ventricular ejection fraction, anemia	Placebo	IV ferric carboxymaltose	Intravenous ferric carboxymaltose or saline placebo was administered every 3 weeks.	Italy	2 weeks	Yes	Increase in ferritin, transferrin saturation, hepcidin, and by a reduction of soluble transferrin receptor.	– consecutive clinically stable patients with chronic HF that presented a left ventricular ejection fraction ≤45%, – anemia (haemoglobin 9–12 g/dl in women or 9–13 g/dl in men) – iron deficiency (serum ferritin <100 µg/L).	Chemoreflex sensitivity cardiorespiratory sleep study, symptom assessment and cardiopulmonary exercise test
[27]	Charles-Edwards et al. (FERRIC-HF II)	2019	Patients of chronic Heart Failure and Iron Deficiency	Placebo	Iron Isomaltoside	Iron (III) isomaltoside 1000 added to 100 mL of sterile 0.9% saline for infusions.	United Kingdom	2 Weeks	Yes	-	– Age ≥30 years – stable symptomatic chronic HF (New York Heart Association [NYHA] III and left ventricular ejection fraction [LVEF] ≤45%. – Use of optimal HF drugs for ≥4 weeks without dose changes.	The primary end point of PCr t 1/2 at 2 weeks.
[28]	Karla et al. (IRONMAN)	2022	Patients of heart failure with iron deficiency	Usual care	Intravenous ferric derisomaltose	Intravenous ferric derisomaltose doses depend on the bodyweight range	United Kingdom	2·7 years (IQR 1·8–3·6).	Yes	Ferritin level of <100 ng per milliliter or a level of 100 to 300 ng per milliliter with a transferrin saturation of <20%)	– Adults ≥18 years with heart failure – left ventricular ejection fraction of 40% or less. – hemoglobin level greater than 9.0 g per deciliter.	All hospital admissions for heart failure and cardiovascular death
[11]	Mentz et al. (HEART-FID)	2023	Patients of Heart Failure with Iron Deficiency	Placebo	IV Ferric Carboxymaltose	Dosing was weight-based; two doses separated by 7 days.	USA, Canada, NewZealand	12 months	Yes	Iron deficiency (serum ferritin <100 µg/L or transferrin saturation <20%)	– Aged 18 years or older, with new or established symptomatic heart failure, – Evidence of iron deficiency – Left ventricular ejection fraction of 45%.	Recurrent hospital admissions for heart failure and cardiovascular death.
[29]	Martens et al. (IRON-CRT)	2021	Symptomatic HFrEF patients with iron deficiency and a persistently reduced left ventricular ejection fraction <45%)	Standard care	IV Ferric Carboxymaltose	Calculated based on screened weight.	Belgium	3 months	Yes	Defined as a serum ferritin <100 ng/mL or serum ferritin between 100 and 300 ng/mL if transferrin saturation (TSAT) was <20%.	– Aged >_18 years, (ii) had – Stable heart failure at least 4 weeks – Received CRT as part of their treatment plan for HFrEF – Association (NYHA) class >_II – Had iron deficiency	Change in LVEF from baseline to 3-month.
[19]	Marcusohn et al.	2022	Patients With Iron Deficiency Hospitalized due to Acute Heart Failure	Optimal medical therapy	IV Sodium Ferric Gluconate Complex	Patients in the treatment group received 3–5 doses of IV SGFC 125 mg.	Palestine	24 weeks	Yes	Ferritin levels ,100 ng/mL or ferritin 100–300 ng/mL with transferrin saturation ,20%.	– Hemoglobin levels of 8–14 mg/dL on admission. – Ferritin levels ,100 ng/mL or ferritin 100–300 ng/mL with transferrin saturation – N-terminal pro–B-type natriuretic peptide (NT-proBNP) level 300 pg/mL. – Treatment with IV loop diuretics.	Change in distance during a 6-minute walk test (6MWT) from baseline to 12 and 24 weeks after initial assessment.

Table 2. Baseline characteristics, continuous data is reported as mean(SD) for FE/Control; and categorical data is reported as event (Total) for FE/Control.

Study	Nunez et al.	Okonko et al.	Ponikowski et al.	Toblli et al.	Ponikowski et al	Veldhuisen et al.	Yeo et al.	Anker et al. (FAIR-HF)	Caravita et al.	Charles-Edwards et al. (FERRIC-HF II)	Karla et al. (IRONMAN)	Mentz et al. (HEART-FID)	Martens et al. (IRON-CRT)	Marcusohn et al.
Year	2020	2008	2015	2007	2021	2017	2018	2009	2022	2019	2022	2023	2021	2022
Race (FE/Control)		Caucasian 21(88%)	White 149(99%)		White 528 Asian 26 Other 4		Chinese 10 Indian 3 Malay 10 Other 1	White 303(99.7%)		White 17(81%)	White 519 Black 12 Asian 35 Other 3	White 1324 Black 162 Asian 19 Other 27		
		Caucasian 10(91%)	White 150(99%)		White 523 Asian 22 Other 5		Chinese 15 Indian 4 Malay 6 Other 0	White 155(100%)		White 14(74%)	White: 524, Black: 7, Asian: 31, Other: 6	White: 1325, Black: 160, Asian: 21, Other: 27		
NYHA	II , III	II , III	II , III		I, II, III, IV	II , III		II , III	III	III	II, III, IV	II, III, IV	II, III	
N	27/26	24/11	150/151	20/20	558/550	86/86	24/25	304/155	38/20	21/19	569/568	1532/1533	37/38	18/16
Age	71.5(10.2)/ 72.3(9.4)	64(14)/ 62(11)	68.8(9.5)/ 69.5(9.3)	76(7)/ 74(8)	71.2(10.8)/ 70.9(11.1)	63(12)/ 64(11)	61.1(10.8)/ 64(10)	67.8(10.3)/ 67.4(11.1)	71(10)/ 71(10)	70(12)/62(13)	73.33(9.96)/ 73.23(8.92)	68.6(10.9)/ 68.6(11.2)	72(12)/ 73(9)	70.07(9.81)/ 75.67(9.75)
Male	21(27)/ 19(26)	17(24)/ 8(11)	83(150)/ 77(151)		314(558)/ 300(550)	60(86)/ 69(86)	18(24)/ 20(25)	145(304)/ 70(155)	30(38)/ 16(20)	16(21)/13(19)	427(569)/ 410(568)	1026(1532)/ 1002(1533)	26(37)/ 25(38)	12(18)/ 11(16)
BMI (kg/m2)		26(5)/ 28(5)	28.3(4.6)/ 29.1(5.7)	28.7(3.3)/ 29(3.4)	28.1(5.6)/ 28(5.7)	27.5(5)/ 26.9(4.4)28		28(4.8)/ 28.1(5.1)	26(4.6)/ 26.8(5.2)	29(4)/30(7)	28.6(5.9)/ 28.5(5.8)		27(5)/ 27(5)	
NYHA II	24(27)/ 26(26)	13(24)/ 6(11)	80(150)/ 91(151)		255(558)/ 240(550)	61(86)/ 54(86)		53(304)/ 29(155)		9(21)/10(19)	328(569)/ 320(568)	797(1532)/ 820(1532)	22(37)/ 19(38)	
NYHA III	3(27)/ 0(26)	11(24)/ 5(11)	70(150)/ 60(151)		272(558)/ 277(550)	25(86)/ 32(86)		251(304)/ 126(155)			230(569)/ 238(568)	711(1532)/ 692(1532)	15(37)/ 19(38)	
NYHA IV					16(558)/ 22(550)						11(569)/ 10(568)	22(1532)/ 19(1532)		
NT-proBNP (pg/mL)	1932(1451.1)/ 1630(1299.6)		2511(5006)/ 2600(4555)	255.9(124.6)/ 267.5(114.9)	5217.3(3974)/ 5388(4392.7)	1576/ 1469				1261.7(1438.2)/ 507.67(519.83)		1752.4(1842.4)/ 1672.5(1612.9)	1831(2057.6)/ 1525(1107.1)	
BNP (pg/mL)			772(995)/ 770(995)		1195(678.6)/ 1320.7(856.3)	491/ 460			638(798)/ 549(490)					8707.7(7689.8)/ 4166.7(4199)
LVEF	39.5(9)/ 37.3(8.6)	30(7)/ 29(6)	37.1(7.5)/ 36.5(7.3)		32.6(9.6)/ 32.7(10)	33(9)/ 31(8)	38.8(17.5)/ 33.2(14.8)	31.9(5.5)/ 33(6.1)	35(7)/ 35(8)	37(8)/ 37(8)	31.33(8.9)/ 33(8.9)	30.8(7)/ 30.6(7.3)	33(8)/ 34(7)	31.67(28.15)/ 42(24.38)
6-min walk test distance	272(48.5)/ 281(89.41)		288(98)/ 302(97)	192.3(60.9)/ 190.7(56.1)			252.4(122.7)/ 242.6(66.8)	274(105)/ 269(109)		324(79)/ 313(67)		273.9(109.7)/ 274.7(109.4)		216.3(82.78)/ 224.93(96.56)
SBP (mm Hg)	119.3(18.)/ 128(25.9)	120(22)/ 116(18)	125(14)/ 124(13)	139.7(8.2)/ 138.8(8.3)	119.8(15.2)/ 119.7(15.6)			126(15)/ 126(15)		124(16)/ 122(17)	119(19.3)/ 119.33(20.1)		121(15)/ 115(15)	
DBP (mm Hg)		69(9)/ 70(9)	75(8)/ 75(8)	74.4(9.6)/ 73.4(7.5)	72.6(10.3)/ 71.9(9.9)			77(9)/ 76(10)		73(10)/ 71(14)				
Hemoglobin (g/dl)	12.8(1.2)/ 13.6(1.49)	12.6(1.2)/ 12.2(1)	12.37(141)/ 12.42(1.3)	10.3(0.6)/ 10.2(0.5)	12.3(1.6)/ 12·1(1.6)	12.9(1.3)/ 13(1.5)	11.6(1.9)/ 13.1(1.3)	11.9(1.3)/ 11.9(1.4)		130(15)/ 128(20)	12.(1.2)/ 12.1(1.3)	12.6(1.4)/ 12.5(1.4)	13.3(1.2)/ 13.1(1.3)	11.73(1.36)/ 11.36(1.86)
Serum ferritin	85(54.8)/ 61.6(71.3)	62(37)/ 88(62)	57(48.4)/ 57.1(41.6)	73(29.9)/ 70.6(21.4)	83·9(62.2)/ 88.5(68.6)	48/ 53	91.4(80.4)/ 84.1(63.7)	52.5(54.5)/ 60.1(66.5)		34(25.5)/ 59(32.04)	55(41.6)/ 55(40.9)	56(47.3)/ 57.3(51.4)	75.3(52.4)/ 74.3(43.1)	102.33(92.5)/ 114(99.2)
Transferrin saturation	15.6(5.6)/ 15(8.2)	20(8)/ 21(9)	20.2(17.6)/ 18.2(8.1)	0.2(0.01)/ 0.2(0.01)	15.2(8.3)/ 14.2(7.5)	17.3/ 18.1	15.7(10.1)/ 13.9(6.8)	17.7(12.6)/ 16.7(8.4)		21(8)/ 18(10)	15.3(6.7)/ 14.7(6.7)	23.9(11.2)/ 23(10.3)	18.8(6)/ 19.4(7)	12.3(4.3)/ 13.6(5.4)
Ferritin <100 ng/mL			136(150)/ 133(151)		408(558)/ 380(550)									
eGFR <60 mL/min per 1·73 m²					292(558)/ 288(550)							288(801)/ 278(752)		

Study	Nunez et al.	Okonko et al.	Ponikowski et al.	Toblli et al.	Ponikowski et al	Veldhuisen et al.	Yeo et al.	Anker et al. (FAIR-HF)	Caravita et al.	Charles-Edwards et al. (FERRIC-HF II)	Karla et al. (IRONMAN)	Mentz et al. (HEART-FID)	Martens et al. (IRON-CRT)	Marcusohn et al.
Year	2020	2008	2015	2007	2021	2017	2018	2009	2022	2019	2022	2023	2021	2022
eGFR mL/min per 1·73 m²	60.2(16.7)/ 64.1(23.8)		66.4(21.7)/ 63.5(209)					63.8(21.2)/ 64.8(25.3)			57.2(12.2)/ 52.2(22.9)		56(25)/ 51(22)	
Atrial fibrillation	10(27)/ 14(26)		66(150)/ 73(151)		314(558)/ 305(550)	35(86)/ 41(86)		94(304)/ 44(155)	8(38)/ 7(20)	6(21)/ 4(19)	284(569)/ 250(568)	223(676)/ 240(664)		10(18)/ 5(16)
ACS			90(150)/ 90(151)		229(558)/ 213(550)	58(86)/ 55(86)	12(24)/ 13(25)		27(38)/ 13(20)		292(569)/ 285(568)			
Hypertension	22(27)/ 16(26)	12(24)/ 5(11)	130(151)/ 130(151)	2(20)/ 3(20)	468(558)/ 471(550)	62(86)/ 56(86)	21(24)/ 18(25)	243(304)/ 128(155)		13(21)/ 13(19)	297(569)/ 315(568)		32(37)/ 37(38)	17(18)/ 15(16)
Diabetes	15(27)/ 14(26)	8(24)/ 4(11)	38(150)/ 45(151)		227(558)/ 243(550)	26(86)/ 32(86)	15(24)/ 15(25)	93(304)/ 37(155)		10(21)/ 10(19)	252(569)/ 269(568)	246(694)/ 264(691)	17(37)/ 19(38)	11(18)/ 12(16)
CKD	8(27)/ 7(26)				222(558)/ 227(550)							178(424)/ 191(400)		12(18)/ 12(16)
Anemia	10(27)/ 6(26)				292(558)/ 312(550)					11(21)/ 9(19)		306(858)/ 339(900)		
Dyslipidaemia	18(27)/ 16(26)	7(24)/ 5(11)	98(150)/ 98(151)		300(558)/ 292(550)		20(24)/ 20(25)	144(304)/ 70(155)		7(21)/ 7(19)				16(18)/ 14(16)
ICD					67(558)/ 64(550)	25(86)/ 33(86)			23(38)/ 16(20)		91(569)/ 72(568)	495(1532)/ 484(1532)		
CRT					33(558)/ 30(550)	11(86)/ 11(86)			12(38)/ 6(20)		125(569)/ 118(568)	230(1532)/ 232(1532)	23(37)/ 19(38)	
ACE inhibitor/ ARB						81(86)/ 77(86)		281(304)/ 141(155)		16(21)/ 17(19)		901(1532)/ 923(1530)	34(37)/ 33(38)	
Sacubitril–valsartan	10(27)/ 8(26)										130(569)/ 110(568)	461(1532)/ 448(1532)		
ACE inhibitor	7(27)/ 6(26)	18(24)/ 8(11)	116(150)/ 118(151)	19(20)/ 20(20)	293(558)/ 283(550)		11(24)/ 8(25)		21(38)/ 10(20)		271(569)/ 281(568)			
ARB	5(27)/ 4(26)	5(24)/ 2(11)	34(150)/ 37(151)	5(20)/ 4(20)	97(558)/ 100(550)		8(24)/ 5(25)		12(38)/ 5(20)		90(569)/ 113(568)			
Beta-blocker	25(27)/ 21(26)	20(24)/ 11(11)	133(150)/ 139(151)	20(20)/ 20(20)	453(558)/ 461(550)	84(86)/ 85(86)	24(24)/ 20(25)	262(304)/ 129(155)	34(38)/ 20(20)	18(21)/ 16(19)	500(569)/ 509(568)	1415(1532)/ 1418(1532)	37(37)/ 37(38)	
antimineralocorticoid		11(24)/ 6(11)			376(558)/ 352(550)	58(86)/ 62(86)	7(24)/ 10(25)		20(38)/ 11(20)		325(569)/ 307(568)	858(1532)/ 847(1532)	30(37)/ 29(38)	
Digoxin	1(27)/ 4(26)	6(24)/ 2(11)	29(150)/ 40(151)	13(20)/ 12(20)	83(558)/ 101(550)			46(304)/ 25(155)		6(21)/ 4(19)	70(569)/ 65(568)			
SGLT2 inhibitor											15(569)/ 14(568)	118(1532)/ 111(1532)		
Loop diuretic	25(27)/ 24(26)		132(150)/ 139(151)		483(558)/ 465(550)		21(24)/ 23(25)		34(38)/ 15(20)	14(21)/ 12(19)	458(569)/ 468(568)		20(37)/ 21(38)	
Insulin			18(150)/ 20(151)					27(304)/ 9(155)			80(569)/ 101(568)			
Other Glucose loweing medication								49(304)/ 22(155)	18(38)/ 9(20)		223(569)/ 239(568)			

Abbreviations: ACE-angiotensin-converting enzyme; ACS-acute coronary syndrome; CKD-chronic kidney disease; CRT-cardiac resynchronization therapy; DBP-diastolic blood pressure ; ICD-implantable cardioverter-defibrillator; LVEF-left ventricle ejection fraction; SBP-systolic blood pressure; SGLT2-sodium-glucose transport protein 2.
reported as median

Table 3. GRADE evidence profile.

Certainty assessment							Summary of findings				
Participants (studies) Follow-up	Risk of bias	Inconsis-tency	Indirect-ness	Impre-cision	Publication bias	Overall certainty of evidence	Study event rates (%)		Relative effect (95% CI)	Anticipated absolute effects	
							With Placebo or Standard Care	With IV Iron		Risk with Placebo or Standard Care	Risk difference with IV Iron
Cardiovascular Mortality											
6145 (6 RCTs)	not serious	not serious	not serious	not serious	none	⊕⊕⊕⊕ High	509/2994 (17.0%)	462/3151 (14.7%)	***RR 0.90*** *(0.80 to 1.01)*	170 per 1,000	***17 fewer per 1,000*** *(from 34 fewer to 2 more)*
All cause mortality											
5281 (8 RCTs)	not serious	not serious	not serious	not serious	none	⊕⊕⊕⊕ High	376/2557 (14.7%)	336/2724 (12.3%)	***RR 0.88*** *(0.78 to 1.01)*	147 per 1,000	***18 fewer per 1,000*** *(from 32 fewer to 1 more)*
Hospital admission for heart failure (first event)											
2813 (5 RCTs)	not serious	not serious	not serious	not serious	none	⊕⊕⊕⊕ High	451/1326 (34.0%)	393/1487 (26.4%)	***RR 0.85*** *(0.77 to 0.95)*	340 per 1,000	***51 fewer per 1,000*** *(from 78 fewer to 17 fewer)*
Hospital admission for heart failure (total events)											
5978 (7 RCTs)	not serious	not serious	not serious	not serious	none	⊕⊕⊕⊕ High	877/2912 (30.1%)	707/3066 (23.1%)	***RR 0.80*** *(0.73 to 0.86)*	301 per 1,000	***60 fewer per 1,000*** *(from 81 fewer to 42 fewer)*
CVD death and hospital admission for heart failure (number of events) rater per 100 patient year											
2704 (3 RCTs)	not serious	not serious	not serious	not serious	none	⊕⊕⊕⊕ High	799/1273 (62.8%)	649/1431 (45.4%)	***RR 0.79*** *(0.74 to 0.85)*	628 per 1,000	***132 fewer per 1,000*** *(from 163 fewer to 94 fewer)*
Hospital admission for heart failure (number of events) rater per 100 patient year											
2704 (3 RCTs)	not serious	not serious	not serious	not serious	none	⊕⊕⊕⊕ High	617/1273 (48.5%)	478/1431 (33.4%)	***RR 0.76*** *(0.70 to 0.83)*	485 per 1,000	***116 fewer per 1,000*** *(from 145 fewer to 82 fewer)*
6-min. walk distance at follow up											
4820 (8 RCTs)	not serious	very serious[a]	not serious	not serious	none	⊕⊕◯◯ Low	2341	2479	-	*The mean 6-min. walk distance at follow up was **0***	*MD **23.56 higher** (21.42 higher to 25.71 higher)*
Change in 6-min. walk distance from baseline											
3865 (4 RCTs)	not serious	not serious	not serious	not serious	none	⊕⊕⊕⊕ High	1858	2007	-	*The mean change in 6-min. walk distance from baseline was **0***	*MD **2.34 higher** (0.69 higher to 4 higher)*
Any adverse effect											
343 (2 RCTs)	not serious	not serious	not serious	very serious[b]	none	⊕⊕◯◯ Low	116/170 (68.2%)	124/173 (71.7%)	***RR 1.06*** *(0.94 to 1.20)*	682 per 1,000	***41 more per 1,000*** *(from 41 fewer to 136 more)*
Any seriious adverse event											
2748 (7 RCTs)	not serious	not serious	not serious	not serious	none	⊕⊕⊕⊕ High	771/1363 (56.6%)	707/1385 (51.0%)	***RR 0.91*** *(0.85 to 0.97)*	566 per 1,000	***51 fewer per 1,000*** *(from 85 fewer to 17 fewer)*
Any adverse event leading to withdrawal											
344 (2 RCTs)	not serious	not serious	not serious	very serious[b]	none	⊕⊕◯◯ Low	19/171 (11.1%)	14/173 (8.1%)	***RR 0.74*** *(0.38 to 1.42)*	111 per 1,000	***29 fewer per 1,000*** *(from 69 fewer to 47 more)*
Abnormal lab test, vital sign or physical finding											
763 (2 RCTs)	not serious	not serious	not serious	very serious[b]	none	⊕⊕◯◯ Low	2/306 (0.7%)	1/457 (0.2%)	***RR 0.50*** *(0.05 to 5.46)*	7 per 1,000	***3 fewer per 1,000*** *(from 6 fewer to 29 more)*

CI: confidence interval; MD: mean difference; RR: risk ratio
Explanations:
a. I square test > 90%
b. Low number of events < 300 events.

Primary Outcomes

Overall analysis

A. Cardiovascular mortality

There was an insignificant risk ratio between the IV iron group and control (RR = 0.90, 95% CI 0.80 to 1.01; p = 0.07, n = 7), with no heterogeneity (I^2 = 0%, p = 0.84) **(Figure 3a and Figure 4).**

B. All-cause mortality

There was an insignificant risk ratio between the IV iron group and control (RR = 0.88, 95% CI 0.78 to 1.01; p = 0.06, n = 6), with no heterogeneity (I^2 = 0%, p = 0.47) **(Figure 3b and Figure 4).**

C. Hospital admission for heart failure (first event)

There was a significant risk ratio between the IV iron group and control (RR = 0.92, 95% CI 0.84 to 1.00; p = 0.04, n = 3), with moderate heterogeneity (I^2 = 44%, p = 0.12) (**Figure 3c and Figure 4**). Heterogeneity reduced when excluding Mentz et al., and results remained significant in favor of IV iron (RR = 0.85, 95% CI 0.77 to 0.95; p = 0.004), with moderate heterogeneity (I^2 = 29%, p = 0.23).

D. Hospital admission for heart failure (total event)

IV iron group had lower hospital admissions than the control group (RR = 0.78, 95% CI 0.72 to 0.85; p < 0.00001, n = 2), with moderate heterogeneity (I^2 = 55%, p = 0.03). Heterogeneity was reduced by excluding Ponikowiski et al., and the results remained significant in favor of the IV iron group (RR = 0.80, 95% CI 0.73 to 0.86; p < 0.0001), with moderate heterogeneity (I^2 = 30%, p = 0.20) **(Figure 3d and Figure 4).**

E. Cardiovascular death and hospital admission for heart failure (number of events, rate per 100 patients in a year)

IV iron group was favored over the control group (RR = 0.81, 95% CI 0.76 to 0.87; p < 0.00001, n = 6), with high heterogeneity (I^2 = 63%, p = 0.04). Heterogeneity was reduced by excluding Mentz et al., and the results remained significant in favor of the IV iron group (RR = 0.79, 95% CI 0.74 to 0.85; p < 0.00001, n = 5), with no heterogeneity (I^2 = 0%, p = 0.58) **(Figure 3e and Figure 4).**

F. Hospital admission for heart failure (number of events, rate per 100 patients in a year)

IV iron group was favored over the control group (RR = 0.75, 95% CI 0.68 to 0.81; p < 0.00001, n = 5), with moderate heterogeneity (I^2 = 38%, p = 0.19). Heterogeneity was reduced by excluding Ponikowiski et al., and the results remained significant in favor of the IV iron group (RR = 0.76, 95% CI 0.70 to 0.83; p < 0.00001, n = 4), with no heterogeneity (I^2 = 0%, p = 0.46) **(Figure 3f and Figure 4).**

Subgroup analysis of main outcomes

Subgroup analysis according to chronic or acute heart failure

In cardiovascular mortality, neither acute or chronic conditions showed significant differences with no heterogeneity observed (RR = 0.89, 95% CI 0.78 to 1.01; p = 0.06, n = 1), and (RR = 0.97, 95% CI 0.73 to 1.30; p = 0.85, n = 5), respectively (**Figure S1**).

In all-cause mortality, groups of chronic conditions hovered around significance, while overall results were insignificant (RR = 0.88, 95% CI 0.77 to 1.00; p = 0.05, n = 6), with no heterogeneity (I^2 = 0%, p = 0.55) (**Figure S2**).

For hospital admission for heart failure (first event), the chronic heart failure group showed only significant preferences toward iron after removing HEART-FID due to heterogeneity (RR = 0.85, 95% CI 0.73 to 0.98; p = 0.03, n = 4), heterogeneity (I^2 = 56%, p = 0.1). (**Figure S3**).

In terms of total hospital admission for heart failure, in both acute and chronic conditions, iron was effective in reducing the total events (RR = 0.80, 95% CI 0.72 to 0.89; p < 0.0001, n = 5), heterogeneity (I^2 = 63%, p = 0.02), reduced by removing Ponikowski 2015 (I^2 = 32%, p = 0.21), and (RR = 0.73, 95% CI 0.64 to 0.83; p < 0.001), respectively (**Figure S4**).

Similar results were obtained for both drugs for CVD death and hospital admission for heart failure (number of events) rater per 100 patient-year [chronic heart failure (RR = 0.84, 95% CI 0.77 to 0.92; p < 0.0001, n = 3), heterogeneity (I^2 = 75%, p = 0.02), reduced by removing HEART-FID (I^2 = 0%, p = 0.44); acute heart failure (RR = 0.78, 95% CI 0.70 to 0.86; p < 0.0001, n = 1)], and hospital admission for heart failure (number of events)

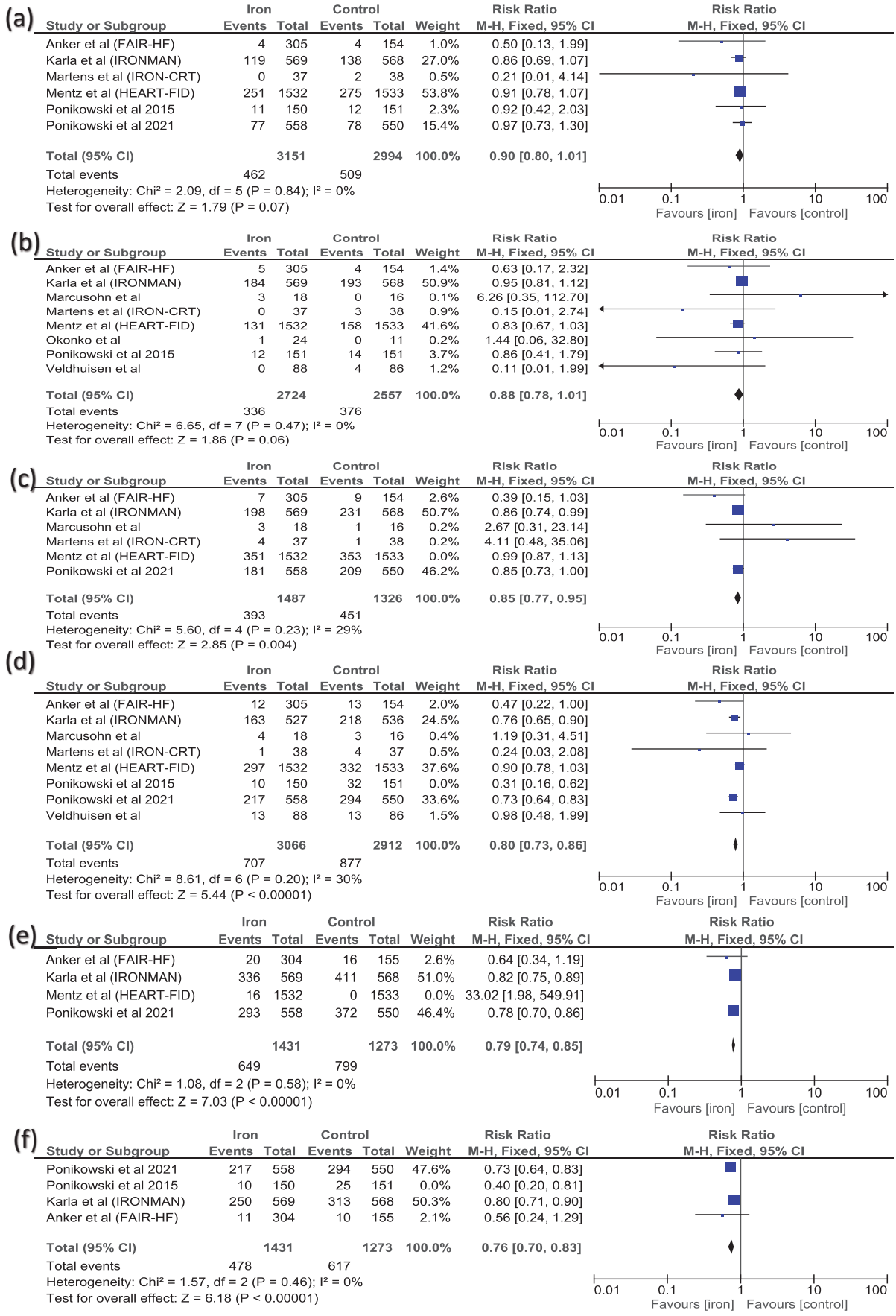

Figure 3. Forest plots examining the cardiovascular outcomes of intravenous iron infusion in patients with heart failure: (a) cardiovascular mortality; (b) all-cause mortality; (c) hospital admission for heart failure (first event); (d) hospital admission for heart failure (total event); (e) cardiovascular death and hospital admission for heart failure (number of events, rate per 100 patients in a year); (f) hospital admission for heart failure (number of events, rate per 100 patients in a year).

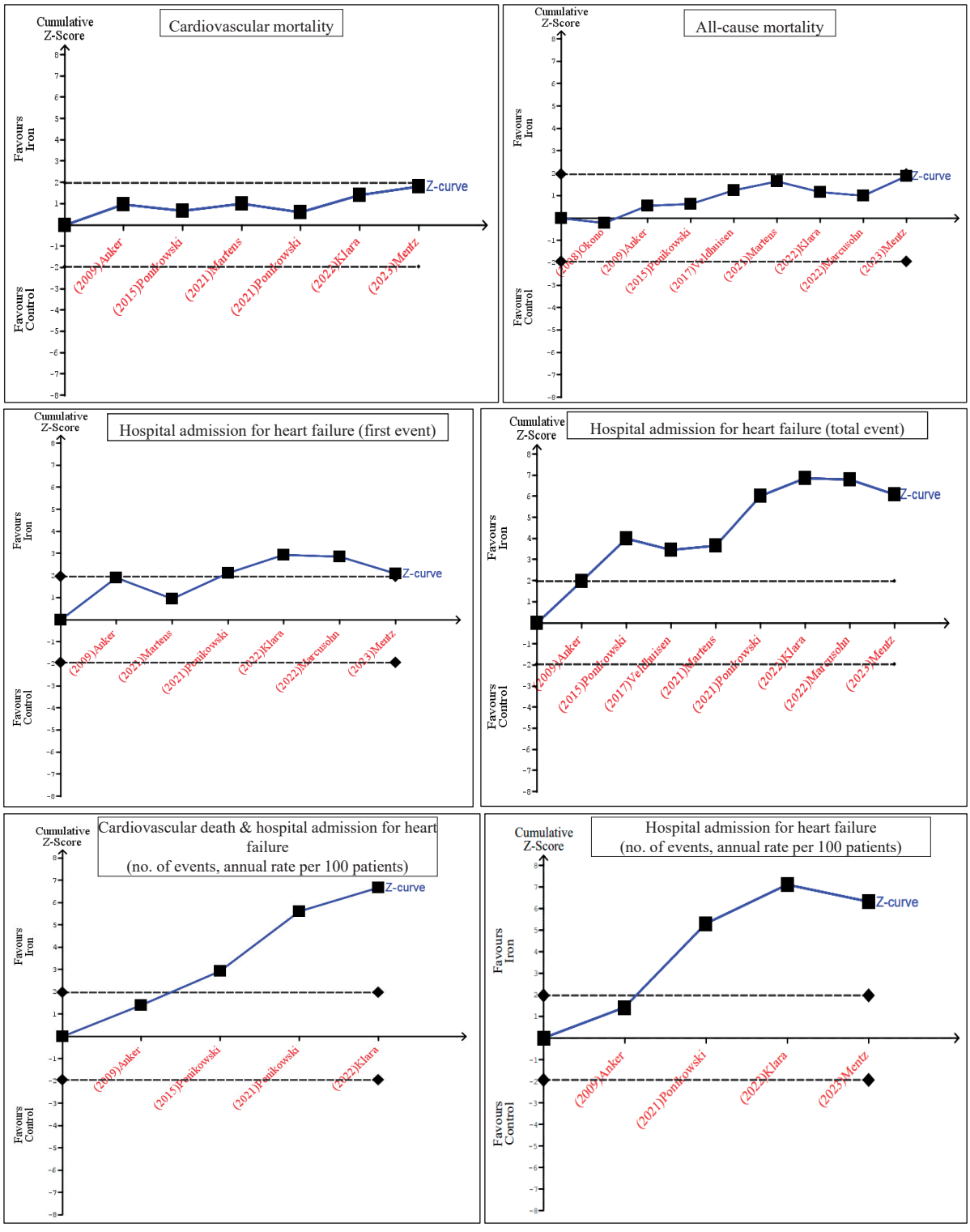

Figure 4. Sequential analysis for the main outcomes, cardiovascular mortality; all-cause mortality; hospital admission for heart failure (first event); hospital admission for heart failure (total event); cardiovascular death and hospital admission for heart failure (number of events, rate per 100 patients in a year); hospital admission for heart failure (number of events, rate per 100 patients in a year).

rater per 100 patient-year [chronic heart failure (RR = 0.76, 95% CI 0.68 to 0.86; p < 0.0001, n = 3), heterogeneity (I^2 = 54%, p = 0.22), reduced by removing Ponikowski 2015 (I^2 = 0%, p = 0.41); acute heart failure (RR = 0.73, 95% CI 0.64 to 0.83; p < 0.0001), n = 2] (**Figure S5 and Figure S6**).

Subgroup analysis according to the iron preparation

In cardiovascular mortality, neither Ferric carboxymaltose nor ferric derisomaltose showed significant preferences with no heterogeneity observed (RR = 0.92, 95% CI 0.80 to 1.05; p = 0.20, n = 5), and (RR = 0.86, 95% CI 0.69 to 1.07; p = 0.17, n = 1), respectively (**Figure S7**).

In all-cause mortality, despite overall results being insignificant, only the ferric carboxymaltose group significantly favored iron over the control (RR = 0.80, 95% CI 0.65 to 0.98; p = 0.03, n = 5), with no heterogeneity (I^2 = 0%, p = 0.50). Ferric derisomaltose and sodium ferric gluconate complex results were insignificant (**Figure S8**).

For hospital admission for heart failure (first event), ferric carboxymaltose (only after removing HEART-FID due to heterogeneity) and ferric derisomaltose showed a significant reduction in hospital admissions (RR = 0.84, 95% CI 0.72 to 0.99; p = 0.03, n = 4), heterogeneity (I^2 = 56%, p = 0.1, and were not reduced after removing HEART-FID, neither influencing the results), and (RR = 0.86, 95% CI 0.74 to 0.99; p = 0.04, n = 3). respectively (**Figure S9**).

In terms of total hospital admission for heart failure, ferric carboxymaltose and ferric derisomaltose results were effective in reducing the total events (RR = 0.78, 95% CI 0.71 to 0.86; p < 0.001, n = 6), heterogeneity (I^2 = 67%, p = 0.01), reduced by removing Ponikowski 2015 (I^2 = 50%, p = 0.09), and (RR = 0.76, 95% CI 0.65 to 0.90; p = 0.001, n = 5), respectively (**Figure S10**).

Similar results were obtained for both drugs for CVD death and hospital admission for heart failure (number of events) rater per 100 patient-year [ferric carboxymaltose (RR = 0.81, 95% CI 0.73 to 0.89; p < 0.001, n = 3), heterogeneity (I^2 = 75%, p = 0.02), reduced by removing HEART-FID (I^2 = 0%, p = 0.54); ferric derisomaltose (RR = 0.82, 95% CI 0.75 to 0.89; p < 0.0001), n = 1], and hospital admission for heart failure (number of events) rater per 100 patient-year [ferric carboxymaltose (RR = 0.73, 95% CI 0.61 to 0.79; p < 0.0001), n = 3, heterogeneity (I^2 = 35%, p = 0.22), reduced by removing Ponikowski 2015 (I^2 = 0%, p = 0.54); ferric derisomaltose (RR = 0.80, 95% CI 0.71 to 0.90; p < 0.0001), n = 2] (**Figure S11 and Figure S12**).

Adverse effects

Overall analysis

A. Cardiac disorder

IV iron group had fewer cardiac disorders than the control group (RR = 0.81, 95% CI 0.76 to 0.87; p < 0.00001, n = 7), with high heterogeneity (I^2 = 66%, p = 0.01). Heterogeneity was reduced by excluding Anker et al., and the results remained significant in favor of the IV iron group (RR = 0.84, 95% CI 0.78 to 0.90; p < 0.00001, n = 6), with no heterogeneity (I^2 = 0%, p = 0.65) (**Figure 5a)**.

B. Gastrointestinal disorder

There were no significant results between the IV iron and control groups (RR = 0.94, 95% CI 0.68 to 1.29; p = 0.69, n = 6), with no high heterogeneity (I^2 = 0%, p = 0.52) **(Figure 5b**).

C. Injection site condition

There were no significant results between the IV iron and control groups (RR = 1.12, 95% CI 0.79 to 1.59; p = 0.56, n = 3), with no high heterogeneity (I^2 = 0%, p = 0.59) (**Figure 5c)**.

D. Infection

There were no significant results between the IV iron and control groups (RR = 0.88, 95% CI 0.73 to 1.07; p = 0.20, n = 2), with no high heterogeneity (I^2 = 0%, p = 0.49) (**Figure 5d)**.

E. Nervous system, disorder

There were no significant results between the IV iron and control groups (RR = 1.16, 95% CI 0.81 to 1.66; p = 0.41, n = 6), with no high heterogeneity (I^2 = 0%, p = 0.86) (**Figure 5e)**.

F. Respiratory, thoracic, or mediastinal disorder

There were no significant results between the IV iron and control groups (RR = 0.76, 95% CI 0.55 to 1.05; p = 0.10, n = 5), with moderate heterogeneity (I^2 = 40%, p = 0.15). Heterogeneity was reduced by excluding Okonko et al., and the results remained insignificant (RR = 0.81, 95% CI 0.58 to 1.12; p = 0.21, n = 4), with low heterogeneity (I^2 = 20%, p = 0.29) (**Figure 5f)**.

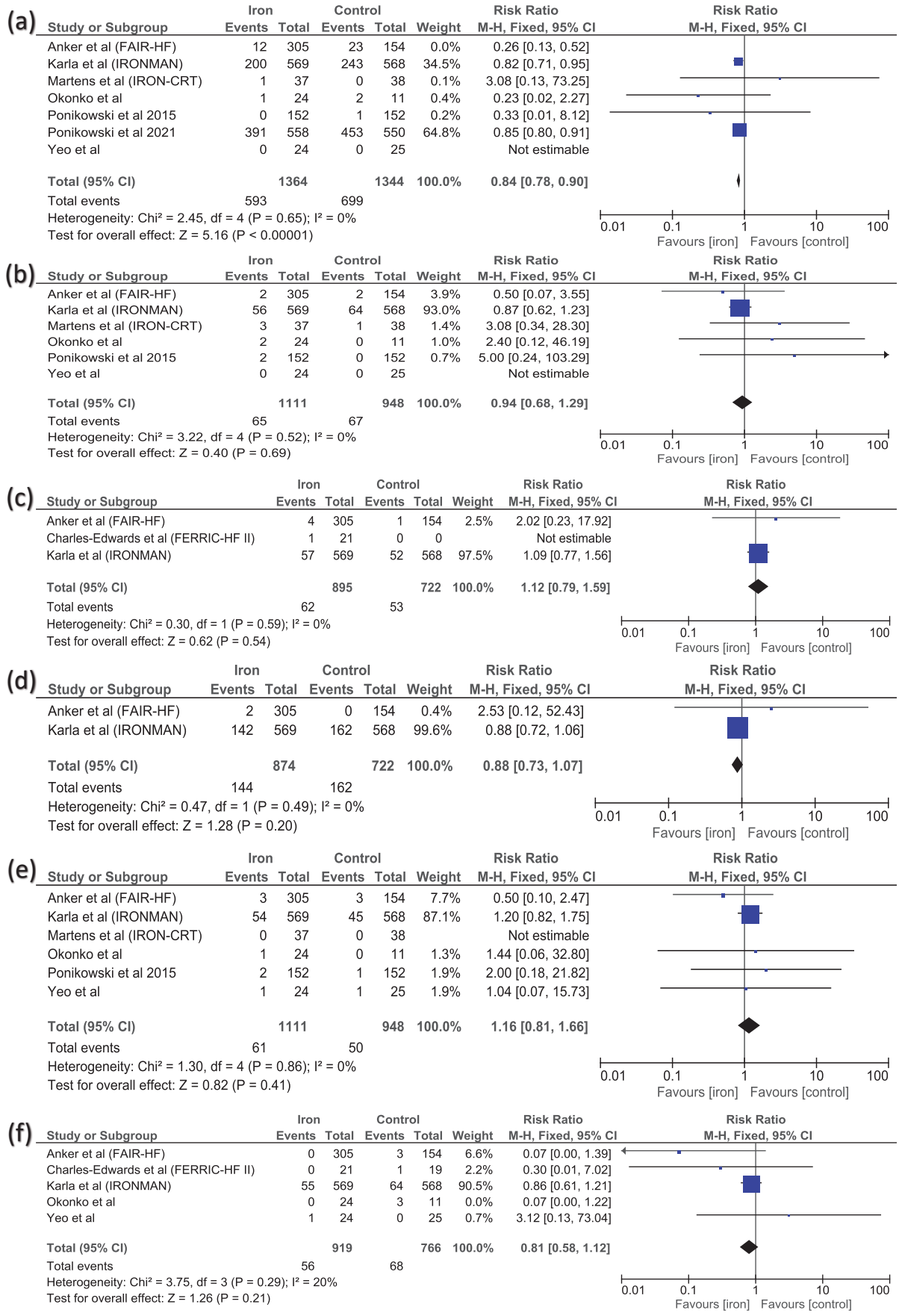

Figure 5. Forest plots examining the adverse effects of intravenous iron infusion in patients with heart failure: (a) cardiac disorder; (b) gastrointestinal disorder; (c) Injection site condition; (d) Infection; (e) nervous system disorder; (f) respiratory, thoracic, or mediastinal disorder.

G. Vascular disorder

There were no significant results between the IV iron and control groups (RR = 0.83, 95% CI 0.55 to 1.27; p = 0.40, n = 4), with no high heterogeneity (I^2 = 0%, p = 0.86) (**Figure 6a**).

H. Any adverse effect

There were no significant results between the IV iron and control groups (RR = 1.09, 95% CI 0.96 to 1.24; p = 0.17, n = 3), with low heterogeneity (I^2 = 25%, p = 0.26). Heterogeneity was reduced by excluding Martens et al. insignificant (RR = 1.06, 95% CI 0.94 to 1.20; p = 0.35, n = 2), with low heterogeneity (I^2 = 0%, p = 0.38) (**Figure 6b**).

I. Any serious adverse event

There were no significant results between the IV iron and control groups (RR = 0.98, 95% CI 0.93 to 1.03; p = 0.37, n = 8), with moderate heterogeneity (I^2 = 54%, p = 0.06). Heterogeneity was reduced by excluding Mentz et al., and the results were altered in favor of IV iron group (RR = 0.91, 95% CI 0.85 to 0.97; p = 0.003, n = 7), with no heterogeneity (I^2 = 0%, p = 0.66) **(Figure 6c)**.

J. Any adverse event leading to withdrawal

There were no significant results between the IV iron and control groups (RR = 0.74, 95% CI 0.38 to 1.42; p = 0.36, n = 2), and heterogeneity analysis was not applicable (**Figure 6d)**.

K. Abnormal lab test, vital signs, or physical finding

There were no significant results between the IV iron and control groups (RR = 0.50, 95% CI 0.05 to 5.46; p = 0.57, n = 2), and heterogeneity analysis was not applicable (**Figure 6e)**.

Subgroup analysis for the adverse effects

Subgroup analysis according to chronic or acute heart failure

Concerning the adverse events, iron reduced cardiac disorders in chronic and acute heart failure despite the lack of studies on the latter (**Figure S13**). Also, iron effectively reduced the serious adverse events in acute heart failure (RR = 0.87, 95% CI 0.77 to 0.99; p = 0.03, n = 6). In the case of chronic heart failure, the results became significant only after removing HEART-FID due to heterogeneity (I^2 = 52%, p = 0.08) that had the highest number of events (RR = 0.93% CI 0.87 to 1.00; p = 0.04, n = 5) without heterogeneity (I^2 = 0%, p = 0.72) (**Figure S14**).

Moreover, regarding respiratory, thoracic or mediastinum disorder, we observed a moderate heterogeneity in the chronic heart failure group (I^2 = 50%, p = 0.11) which was resolved by removing the IRONMAN study and resulted in altering both the overall and chronic heart failure results (RR = 0.23, 95% CI 0.07 to 0.75; p = 0.01, n = 5), low heterogeneity (I^2 = 32%, p = 0.27), and (RR = 0.10, 95% CI 0.02 to 0.54; p = 0.007, n = 4), without heterogeneity (I^2 = 0%, p = 0.75). These results may highlight the importance of iron injections in reducing the serious adverse effects of chronic and acute cases and those related to respiratory, thoracic or mediastinum disorders in chronic cases (**Figure S15**).

No significant differences were observed in acute or chronic conditions in gastrointestinal, nervous system, or vascular disorders (**Figures S16, S17, S18**). It was not possible to subgroup these outcomes: injection site condition, infection, any adverse effect, any adverse event leading to withdrawal, abnormal lab tests, and vital signs or physical findings.

Subgroup analysis according to the iron preparation

Concerning the adverse events, both ferric carboxymaltose and ferric derisomaltose showed a beneficial effect in reducing the cardiac disorder (RR = 0.81, 95% CI 0.76 to 0.87; p < 0.0001, n = 4), and (RR = 0.82, 95% CI 0.71 to 0.95; p = 0.009, n = 1), respectively. Heterogeneity was resolved in the ferric carboxymaltose group by removing FAIR-HF without altering the results. Iron sucrose did not show any significance regarding cardiac disorders; however, it included only one study with an overall small sample size (**Figure S19**).

Moreover, in terms of the presence of any serious adverse effect, despite the insignificant results overall, that was altered when we removed HEART-FID, which resulted in making only ferric carboxymaltose (compared with derisomaltose, iron isomaltoside, and iron sucrose) ferric shows significant reduction (RR = 0.86, 95% CI 0.77 to 0.97; p = 0.01, n = 5) with no heterogeneity. Hence, overall results also became significant (RR = 0.91, 95% CI 0.85 to 0.97; p = 0.009, n = 8) (**Figure S20**).

All formulation results were comparable, and they did not significantly influence gastrointestinal disorder, injection site condition, infection, nervous system disorder, respiratory, thoracic or mediastinum disorder, vascular disorder, any adverse effect, any adverse event leading to withdrawal and abnormal lab test, vital sign or physical finding (**Figures S20–S29**).

Discussion

The current body of evidence shows that IV ferric carboxymaltose treatment reduces the risk of hospital admission for the first and total events of HF worsening. Moreover, it is associated with a lower risk of the combination of cardiovascular death and HF hospitalization (number of events,

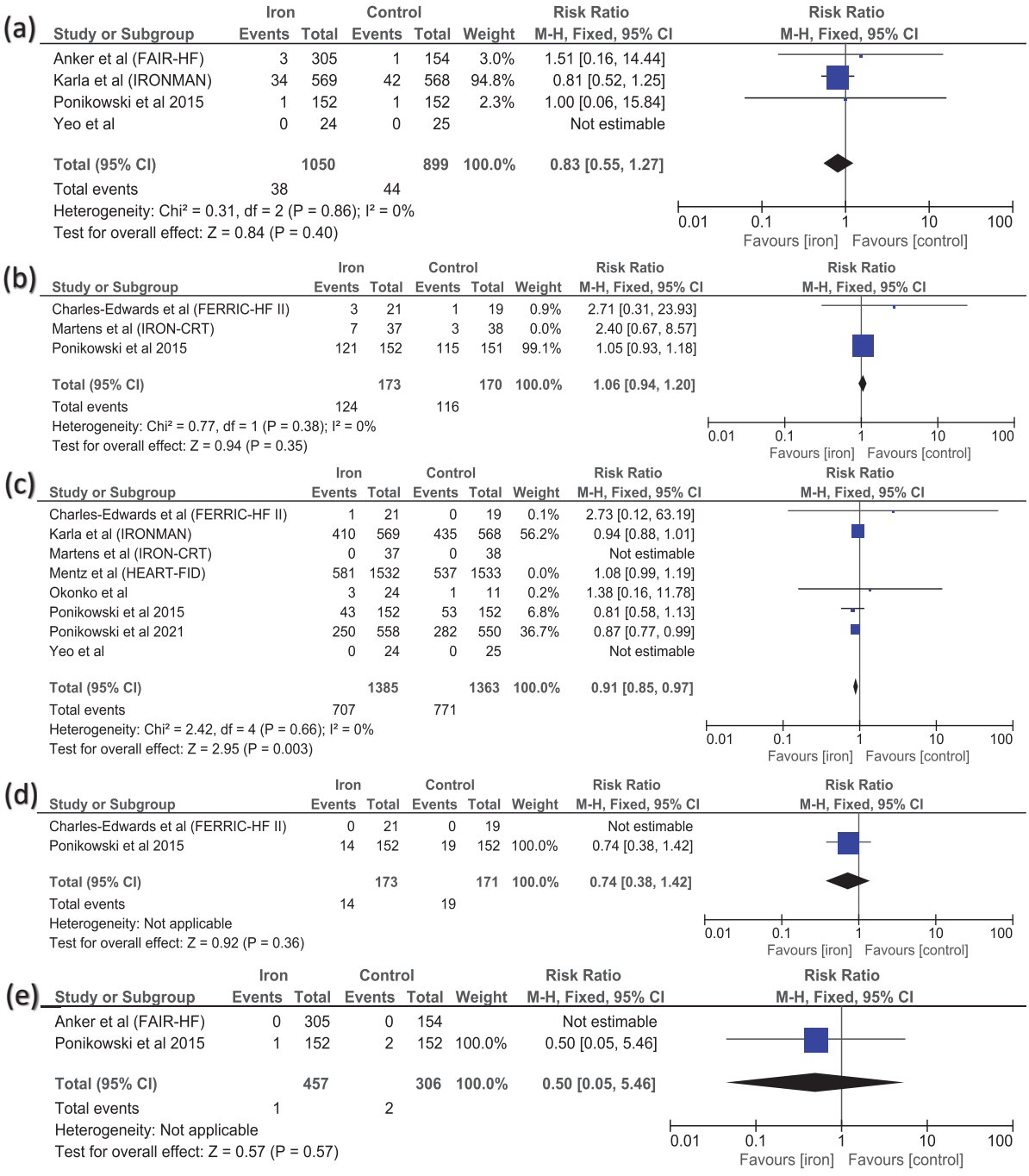

Figure 6. Forest plots examining the adverse effects of intravenous iron infusion in patients with heart failure: (a) vascular disorder; (b) any adverse effect; (c) any serious adverse event; (d) any adverse event leading to withdrawal; (e) abnormal lab tests, vital signs, or physical finding.

rate per 100 patients in a year). However, ferric carboxymaltose does not affect all-cause mortality or cardiovascular mortality. Regarding safety, patients treated with iron therapy experienced fewer cardiac adverse effects than controls. At the same time, they displayed no additional risks of other adverse effects. Our findings confirmed what was previously shown by systematic reviews and meta-analyses, which reported the effectiveness of IV iron supplementation in reducing hospitalization and the combined endpoint of death and hospitalization for decompensated HF [30–32].

Events of acute decompensation are the primary cause of hospital admission for heart failure. According to its mechanism, HF decompensation represents the situation in which neurohormonal signaling, preload, afterload, and intrinsic inotropy are all out of balance, and compensated HF has reached this point [33]. Ventricular filling pressure elevation, venous and arterial congestion, vasoconstriction, and inotropy depression could result from this. Inotropy depression presents clinically as acute symptoms and congestion indicators that necessitate immediate, typically IV, therapy [33]. It has been demonstrated that iron deficiency, whether absolute or functional, can enhance the processes leading to decompensated heart failure by aggravating cardiac congestion, supporting unfavorable cardiac remodeling, and reducing myocardial inotropy. Thus, greater sensitivity to sympathetic stimulation has been observed in iron-deficient mice with cardiac hypertrophy [34]. This may favor peripheral vasoconstriction, a major element in the pathogenesis of HF decompensation by worsening central (i.e., cardiopulmonary) congestion [33].

Iron deficiency may weaken the heart's defenses against oxidative stress because iron is a co-factor for anti-oxidative enzymes. This phenomenon has been linked to the cardiac remodeling process during heart failure [35]. Cardiac remodeling is a deleterious process in HF that leads to cardiac dysfunction with subsequent symptoms of exacerbation [36]. In line with this, experimental evidence has shown that myocardial iron deficiency aggravates acute myocardial ischemia as well as post-ischemic remodeling, which worsens the clinical outcomes of myocardial infarction-associated HF [37]. Moreover, iron deficiency impairs the contractility and relaxation of human cardiomyocytes by downregulating RyR2 channels and suppressing SERCA pump activity [38, 39]. This would then change the inotropy, which would aggravate systolic dysfunction and increase the risk of heart failure decompensation. Conversely, rodents supplemented with IV ferric carboxymaltose had normal Ca2+ signaling again [38]. Therefore, we can assume that iron replacement therapy can reverse the harmful effects of systemic iron deficiency and myocardial iron deficiency on cardiac function in the context of HF, inducing disease control and lesser susceptibility to acute symptomatic events that require hospitalization.

Notably, the latest months have seen progress in research on the impact of IV supplementation in heart failure, which warrants discussion. Further analysis of data from the IRONMAN trial [28] suggested that patients with anemia or with low transferrin saturation (even with adequate ferritin) benefit the most from intravenous iron supplementation [40]. Improved response in patients with low transferrin saturation was also highlighted in a recent meta-analysis by Martens et al. [41]. Furthermore, IRONMAN investigators showed data that indicate a general increase in resilience due to iron supplementation, with effect seen in hospitalizations for both cardiac and non-cardiovascular indications [42]. It is also noteworthy that further evidence for the beneficial influence of intravenous iron in patients with heart failure and preserved ejection fraction emerged from the FAIR-HFpEF trial, which demonstrated a benefit in 6-minute walking test distance [43]. The interplay between ejection fraction and the capacity to utilize and store iron appears as an interesting research topic.

Notably, the combined outcome of cardiovascular mortality and hospitalizations was decreased in the IV iron group, likely due to the reduced odds of HF hospitalization. Nevertheless, iron therapy had no effects on cardiovascular or all-cause mortality. Notably, both AFFIRM-AHF and HEART FID trials showed that supplementation with IV iron does not impact the risk of cardiovascular death. The positive effects of iron therapy in reducing mortality were noted in the IRONMAN study. All of these three studies were conducted during the COVID-19 pandemic, which could have a major influence on the effect of treatment, as the authors disclosed it. Especially in the context of COVID-19, HF population has witnessed a substantial reduction in hospitalizations and an increase in in-hospital mortality [44]. Moreover, in the HEART FID trial, iron

supplementation did not benefit in reducing cardiovascular hospitalizations. Here too, the authors reported possible interference of the COVID-19 pandemic with the treatment outcomes. Further studies outside of COVID-19 are needed to confirm the previous findings [45, 46].

Ferric carboxymaltose demonstrated good tolerability in clinical trials involving patients with iron deficiency. Most adverse events associated with its use were mild to moderate in severity. Commonly reported side effects included headache, dizziness, nausea, abdominal pain, constipation, diarrhea, rash, and injection-site reactions [47]. According to our analysis, we affirm that FC has a good safety profile in HF patients who are iron deficient as it did not increase the risk for any particular side effects relatively. It reduces the risk for cardiovascular side effects. Moreover, we observed that iron injections may reduce any adverse severe event for acute and chronic conditions, and respiratory, thoracic, or mediastinal disorders for chronic conditions mainly. This encourages further investigation of IV ferric carboxymaltose in large-scale studies.

Our study aligens with the newly published meta-analyses, Mhanna et al. conducted a systematic review and meta-analysis analyzing data from 14 RCTs involving 6,614 patients. The study demonstrated that IV iron therapy significantly improved quality of life and the 6-minute walk test compared to standard care, although it did not significantly affect left ventricular ejection fraction. [48] Awad et al. data from 18 RCTs found significant improvements in quality of life, as indicated by Kansas City Cardiomyopathy Questionnaire (KCCQ) scores, and enhanced clinical outcomes, including increased serum ferritin and hemoglobin levels. While all-cause hospitalizations and heart failure-related deaths showed no significant difference, IV iron therapy reduced hospitalizations due to heart failure. [49] Sephien et al. found that IV iron therapy was associated with a significant improvement in quality of life and a notable reduction in first heart failure hospitalizations. However, there was no significant change in all-cause mortality [50].

Implications for future research and clinical practice

The transition from inpatient to outpatient care is a vulnerable period for HF patients, particularly the elderly and those with comorbidities [51]. Additionally, HF hospitalization is associated with an elevated risk of mortality [52]. From an economic standpoint, HF hospitalizations are considered costly, with mean HF-specific inpatient costs in the USA ranging from $10,737 to $17,830 per hospitalization [53].

While recent HF treatments have demonstrated mortality reduction benefits, their impact on hospitalization rates remains neutral [51]. Preventing iron deficiency through iron supplementation can mitigate the risk of HF-related hospitalizations. Notably, IV iron therapy is safe and effective for HF patients, irrespective of anemia. This is because iron deficiency in HF patients can be functional, selectively affecting the myocardium—a condition known as myocardial iron deficiency, which is challenging to diagnose. Consequently, even patients with normal iron levels may benefit from iron therapy.

Therefore, it may become an integral part of routine treatment strategies aimed at preventing decompensation events. However, the promising benefits of IV iron supplementation in patients with HF must be carefully weighed against the potential safety concerns associated with iron overload [54]. IV iron administration introduces substantial amounts of non-transferrin-bound iron, bypassing hepatic regulatory mechanisms, which can lead to iron overload. Most published studies have utilized IV iron sucrose (with a maximum dose of 200 mg per session) or ferric carboxymaltose (with a maximum dose of 1000 mg per week) [55]. Due to gut wall edema, oral iron preparations, typically containing Fe^{2} , have been associated with poor absorption, a high incidence of side effects (affecting up to 40% of patients), and the necessity for up to six months of intake to restore iron stores [55].

On the other hand, unlike the IV form, oral iron absorption is tightly regulated by the effects of hepcidin; thereby, it can rarely lead to iron excess [54]. Oral iron can improve cardiac function, as measured by changes in left ventricular ejection fraction, among HF patients with iron deficiency, according to a recent meta-analysis of four RCTs (n = 582 patients); exercise capacity did not significantly increase [56]. Oral iron supplementation is more practical than IV ferric carboxymaltose due to higher availability and cheaper costs, making the former option worthy of greater inves-

tigation [24]. This points to the need for further research comparing the effects of IV and oral iron on HF-related outcomes.

Notably, the European Cardiology Society heart failure guidelines (2023 update; Recommendation Table 5) indicate that IV should be used to reduce the risk of hospitalization and increase quality of life in patients with iron deficiency and symptomatic heart failure with at least mild reduction of ejection fraction [57]. It is supposed that further extension of this recommendation might follow to include patients with preserved ejection fraction or additional comments on symptoms or the optimal way of diagnosing iron deficiency.

Strengths and limitations

This systematic review and meta-analysis represent the most updated study assessing the safety and efficacy profile of IV ferric carboxymaltose among heart failure patients. Similar work was previously conducted by Zhou et al. in 2019 [32] and Osman et al. in 2021 [31]. However, significant studies have been published since then, such as IRONMAN, HEART-FID, and AFFIRM-AHF, phase 3 RCTs. More recently, Reinhold et al. in 2023 [30], explored the effects of IV iron replacement therapy on cardiovascular outcomes in HF patients. Notably, their focus was solely on efficacy outcomes, lacking examination of safety-related outcomes, which are highly interesting. Our study incorporated updated data from 14 RCTs, involving 6,626 patients, some of which were large-scale, multicenter, double-blind studies.

Concerning the limitations, firstly, not all included studies maintained optimal methodological quality, with some being open-label or single-blind and/or having few participants. Secondly, due to incomplete information, we did not assess the impact of iron supplementation on cardiac function-related outcomes such as left ventricular ejection fraction, HF symptoms (e.g., dyspnea), quality of life, and cardiorespiratory performance. Thirdly, the included studies did not achieve the long-term follow-up needed to identify IV iron-based therapy's benefits fully. Fourthly, comparing the effects of different iron-based treatments (e.g., infused doses and used molecules) was impossible. Regarding iron preparation, most of the included studies covered only ferric carboxymaltose and ferric derisomaltose, both showing good efficacy and a comparable safety profile. However, there is a need for additional studies exploring the outcomes of other iron-based supplementations, including iron isomaltoside, iron sucrose, and sodium ferric gluconate complex, as the number of patients who received these treatments in the included studies was very small, hence insufficient to indicate any differences.

Conclusions

IV iron infusion is an effective option to reduce hospitalization episodes and cardiovascular mortality among HF patients. Additionally, it is a safe and well-tolerable treatment that can be given to this group of patients as an adjuvant therapy to traditional medications. Nevertheless, further studies are still required to confirm the clinical advantages of iron-based supplementations in the context of HF.

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List of Abbreviations: heart failure (HF), intravenous (IV), randomized controlled trial (RCT), mean difference (MD), confidence interval (CI), risk ratio (RR).

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Consent for publication: not applicable.

Availability of data and material: all data generated or analysed during this study are included in this published article.

Authors' contributions. MAazm: conceptualization and methodology. AR, OA, AA, IU, MAazm and BA: investigation and data curation. MAzid: formal analysis. YK and MAzid: Writing – Original Draft. BA: Supervision. MT: Project administration. MAazm, MAzid, MT and BA: Writing – Review & Editing. All authors read and approved the final content.

Conflict of interest statement
The authors declare no conflict of interest.

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References

1. Bozkurt B, Coats AJS, Tsutsui H, et al (2021) Universal definition and classification of heart failure: a report of the Heart Failure Society of America, Heart Failure Association of the European Society of Cardiology, Japanese Heart Failure Society and Writing Committee of the Universal Definition of Heart Failure: Endorsed by the Canadian Heart Failure Society, Heart Failure Association of India, Cardiac Society of Australia and New Zealand, and Chinese Heart Failure Association. Eur J Heart Fail 23:352–380. doi: 10.1002/ejhf.2115.
2. GBD 2017 Disease and Injury Incidence and Prevalence Collaborators (2018) Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet 392:1789–1858. doi: 10.1016/S0140-6736(18)32279-7.
3. Savarese G, Becher PM, Lund LH, et al (2023) Global burden of heart failure: a comprehensive and updated review of epidemiology. Cardiovasc Res 118:3272–3287. doi: 10.1093/cvr/cvac013.
4. Alnuwaysir RIS, Hoes MF, van Veldhuisen DJ, et al (2021) Iron Deficiency in Heart Failure: Mechanisms and Pathophysiology. J Clin Med 11:125. doi: 10.3390/jcm11010125.
5. Anand IS, Gupta P (2018) Anemia and Iron Deficiency in Heart Failure. Circulation 138:80–98. doi: 10.1161/CIRCULATIONAHA.118.030099.
6. Maeder MT, Khammy O, dos Remedios C, Kaye DM (2011) Myocardial and systemic iron depletion in heart failure implications for anemia accompanying heart failure. J Am Coll Cardiol 58:474–480. doi: 10.1016/j.jacc.2011.01.059.
7. Zhang H, Jamieson KL, Grenier J, et al (2022) Myocardial Iron Deficiency and Mitochondrial Dysfunction in Advanced Heart Failure in Humans. J Am Heart Assoc 11:e022853. doi: 10.1161/JAHA.121.022853.
8. Klip IT, Comin-Colet J, Voors AA, et al (2013) Iron deficiency in chronic heart failure: an international pooled analysis. Am Heart J 165:575-582.e3. doi: 10.1016/j.ahj.2013.01.017.
9. Ponikowski P, van Veldhuisen DJ, Comin-Colet J, et al (2015) Beneficial effects of long-term intravenous iron therapy with ferric carboxymaltose in patients with symptomatic heart failure and iron deficiency†. Eur Heart J 36:657–668. doi: 10.1093/eurheartj/ehu385.
10. Ponikowski P, Kirwan B-A, Anker SD, et al (2020) Ferric carboxymaltose for iron deficiency at discharge after acute heart failure: a multicentre, double-blind, randomised, controlled trial. Lancet 396:1895–1904. doi: 10.1016/S0140-6736(20)32339-4.
11. Mentz RJ, Garg J, Rockhold FW, et al (2023) Ferric Carboxymaltose in Heart Failure with Iron Deficiency. New England Journal of Medicine 389:975–986. doi: 10.1056/NEJMoa2304968.
12. Page MJ, McKenzie JE, Bossuyt PM, et al (2021) The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 372:n71. doi: 10.1136/bmj.n71.
13. Higgins JP, Thomas J, Chandler J, et al (2019) Cochrane handbook of systematic reviews of interventions. John Wiley & Sons, Ltd, Chichester (UK).
14. Sterne JAC, Savović J, Page MJ, et al (2019) RoB 2: a revised tool for assessing risk of bias in randomised trials. BMJ 366:l4898. doi: 10.1136/bmj.l4898.
15. Schünemann HJ, Oxman AD, Brozek J, et al (2008) Grading quality of evidence and strength of recommendations for diagnostic tests and strategies. BMJ 336:1106–1110. doi: 10.1136/bmj.39500.677199.AE.
16. Guyatt G, Oxman AD, Akl EA, et al (2011) GRADE guidelines: 1. Introduction—GRADE evidence profiles and summary of findings tables. Journal of Clinical Epidemiology 64:383–394. doi: 10.1016/j.jclinepi.2010.04.026.
17. Joanne E McKenzie, Sue E Brennan, Rebecca E Ryan, et al (2023) Chapter 9: Summarizing study characteristics and preparing for synthesis. In: Cochrane Handbook for Systematic Reviews of Interventions. Cochrane.
18. 10.4.3.1 Recommendations on testing for funnel plot asymmetry. https://handbook-5-1.cochrane.org/chapter_10/10_4_3_1_recommendations_on_testing_for_funnel_plot_asymmetry.htm. Accessed 20 Feb 2024.
19. Marcusohn E, Borreda I, Hellman Y, et al (2022) IV Sodium Ferric Gluconate Complex in Patients With Iron Deficiency Hospitalized due to Acute Heart Failure-Investigator Initiated, Randomized Controlled Trial. J Cardiovasc Pharmacol 80:194–196. doi: 10.1097/FJC.0000000000001287.
20. Núñez J, Miñana G, Cardells I, et al (2020) Noninvasive Imaging Estimation of Myocardial Iron Repletion Following Administration of Intravenous Iron: The Myocardial-IRON Trial. J Am Heart Assoc 9:e014254. doi: 10.1161/JAHA.119.014254.
21. Okonko DO, Grzeslo A, Witkowski T, et al (2008) Effect of intravenous iron sucrose on exercise tolerance in anemic and nonanemic patients with symptomatic chronic heart failure and iron deficiency FERRIC-HF: a randomized, controlled, observer-blinded trial. J Am Coll Cardiol 51:103–112. doi: 10.1016/j.jacc.2007.09.036.
22. Toblli JE, Lombraña A, Duarte P, Di Gennaro F (2007) Intravenous iron reduces NT-pro-brain natriuretic peptide in anemic patients with chronic heart failure and renal insufficiency. J Am Coll Cardiol 50:1657–1665. doi: 10.1016/j.jacc.2007.07.029.
23. van Veldhuisen DJ, Ponikowski P, van der Meer P, et al (2017) Effect of Ferric Carboxymaltose on Exercise Capacity in Patients With Chronic Heart Failure and Iron Deficiency. Circulation 136:1374–1383. doi: 10.1161/CIRCULATIONAHA.117.027497.
24. Yeo TJ, Yeo PSD, Hadi FA, et al (2018) Single-dose intravenous iron in Southeast Asian heart failure patients: A pilot randomized placebo-controlled study (PRACTICE-ASIA-HF). ESC Heart Fail 5:344–353. doi: 10.1002/ehf2.12250.
25. Anker SD, Comin Colet J, Filippatos G, et al (2009) Ferric carboxymaltose in patients with heart failure

and iron deficiency. N Engl J Med 361:2436–2448. doi: 10.1056/NEJMoa0908355.
26. Caravita S, Faini A, Vignati C, et al (2022) Intravenous iron therapy improves the hypercapnic ventilatory response and sleep disordered breathing in chronic heart failure. Eur J Heart Fail 24:1940–1949. doi: 10.1002/ejhf.2628.
27. Charles-Edwards G, Amaral N, Sleigh A, et al (2019) Effect of Iron Isomaltoside on Skeletal Muscle Energetics in Patients With Chronic Heart Failure and Iron Deficiency. Circulation 139:2386–2398. doi: 10.1161/CIRCULATIONAHA.118.038516.
28. Kalra PR, Cleland JGF, Petrie MC, et al (2022) Intravenous ferric derisomaltose in patients with heart failure and iron deficiency in the UK (IRONMAN): an investigator-initiated, prospective, randomised, open-label, blinded-endpoint trial. Lancet 400:2199–2209. doi: 10.1016/S0140-6736(22)02083-9.
29. Martens P, Dupont M, Dauw J, et al (2021) The effect of intravenous ferric carboxymaltose on cardiac reverse remodelling following cardiac resynchronization therapy-the IRON-CRT trial. Eur Heart J 42:4905–4914. doi: 10.1093/eurheartj/ehab411.
30. Reinhold J, Burra V, Corballis N, et al (2023) Effects of Intravenous Iron Replacement Therapy on Cardiovascular Outcomes in Patients with Heart Failure: A Systematic Review and Meta-Analysis. J Cardiovasc Dev Dis 10:116. doi: 10.3390/jcdd10030116.
31. Osman M, Syed M, Balla S, et al (2021) A Meta-analysis of Intravenous Iron Therapy for Patients With Iron Deficiency and Heart Failure. Am J Cardiol 141:152–153. doi: 10.1016/j.amjcard.2020.11.025.
32. Zhou X, Xu W, Xu Y, Qian Z (2019) Iron Supplementation Improves Cardiovascular Outcomes in Patients with Heart Failure. Am J Med 132:955–963. doi: 10.1016/j.amjmed.2019.02.018.
33. Njoroge JN, Teerlink JR (2021) Pathophysiology and Therapeutic Approaches to Acute Decompensated Heart Failure. Circulation Research 128:1468–1486. doi: 10.1161/CIRCRESAHA.121.318186.
34. Turner LR, Premo DA, Gibbs BJ, et al (2002) Adaptations to iron deficiency: cardiac functional responsiveness to norepinephrine, arterial remodeling, and the effect of beta-blockade on cardiac hypertrophy. BMC Physiol 2:1. doi: 10.1186/1472-6793-2-1.
35. Martens P (2022) The Effect of Iron Deficiency on Cardiac Function and Structure in Heart Failure with Reduced Ejection Fraction. Card Fail Rev 8:e06. doi: 10.15420/cfr.2021.26.
36. Azevedo PS, Polegato BF, Minicucci MF, et al (2016) Cardiac Remodeling: Concepts, Clinical Impact, Pathophysiological Mechanisms and Pharmacologic Treatment. Arq Bras Cardiol 106:62–69. doi: 10.5935/abc.20160005.
37. Corradi F, Masini G, Bucciarelli T, De Caterina R (2023) Iron deficiency in myocardial ischaemia: molecular mechanisms and therapeutic perspectives. Cardiovasc Res 119:2405–2420. doi: 10.1093/cvr/cvad146.
38. Chung YJ, Luo A, Park KC, et al (2019) Iron-deficiency anemia reduces cardiac contraction by downregulating RyR2 channels and suppressing SERCA pump activity. JCI Insight 4:e125618, 125618. doi: 10.1172/jci.insight.125618.
39. Hoes MF, Grote Beverborg N, Kijlstra JD, et al (2018) Iron deficiency impairs contractility of human cardiomyocytes through decreased mitochondrial function. European Journal of Heart Failure 20:910–919. doi: 10.1002/ejhf.1154.
40. Cleland JGF, Kalra PA, Pellicori P, et al (2024) Intravenous iron for heart failure, iron deficiency definitions, and clinical response: the IRONMAN trial. European Heart Journal 45:1410–1426. doi: 10.1093/eurheartj/ehae086.
41. Martens P, Augusto SN, Mullens W, Tang WHW (2024) Meta-Analysis and Metaregression of the Treatment Effect of Intravenous Iron in Iron-Deficient Heart Failure. JACC: Heart Failure 12:525–536. doi: 10.1016/j.jchf.2023.11.006.
42. Cleland JGF, Pellicori P, Graham FJ, et al (2024) Adjudication of Hospitalizations and Deaths in the IRONMAN Trial of Intravenous Iron for Heart Failure. Journal of the American College of Cardiology 84:1704–1717. doi: 10.1016/j.jacc.2024.08.052.
43. Von Haehling S, Doehner W, Evertz R, et al (2024) Ferric carboxymaltose and exercise capacity in heart failure with preserved ejection fraction and iron deficiency: the FAIR-HFpEF trial. European Heart Journal 45:3789–3800. doi: 10.1093/eurheartj/ehae479.
44. Shoaib A, Van Spall HGC, Wu J, et al (2021) Substantial decline in hospital admissions for heart failure accompanied by increased community mortality during COVID-19 pandemic. Eur Heart J Qual Care Clin Outcomes 7:378–387. doi: 10.1093/ehjqcco/qcab040.
45. Bhatt AS, Moscone A, McElrath EE, et al (2020) Fewer Hospitalizations for Acute Cardiovascular Conditions During the COVID-19 Pandemic. J Am Coll Cardiol 76:280–288. doi: 10.1016/j.jacc.2020.05.038.
46. Hernández-Vásquez A, Visconti-Lopez FJ, Alburqueque-Cruz R, Rojas-Roque C (2023) Hospitalizations and mortality of patients with heart failure in the COVID-19 era in Peru. Journal of Taibah University Medical Sciences 18:186–189. doi: 10.1016/j.jtumed.2022.07.009.
47. Lyseng-Williamson KA, Keating GM (2009) Ferric carboxymaltose: a review of its use in iron-deficiency anaemia. Drugs 69:739–756. doi: 10.2165/00003495-200969060-00007.
48. Mhanna M, Sauer MC, Al-Abdouh A, et al (2024) Intravenous iron therapy for patients with iron deficiency and heart failure: a systematic review and meta-analysis of randomized controlled trials. Baylor University Medical Center Proceedings 37:466–476. doi: 10.1080/08998280.2024.2326387.
49. Awad AK, Abdelgalil MS, Gonnah AR, et al (2024) Intravenous iron for acute and chronic heart failure with reduced ejection fraction (HFrEF) patients with iron deficiency: An updated systematic review and meta-analysis. Clinical Medicine 24:100211. doi: 10.1016/j.clinme.2024.100211.
50. Sephien A, Dayto DC, Reljic T, et al (2024) Efficacy of Intravenous Iron in Patients with Heart Failure with Reduced Ejection Fraction and Iron Defi-

ciency: A Systematic Review and Meta-Analysis of Randomized Control Trials. Am J Cardiovasc Drugs 24:285–302. doi: 10.1007/s40256-024-00635-7.
51. Mesquita ET, Jorge AJL, Rabelo LM, Jr CVS (2017) Understanding Hospitalization in Patients with Heart Failure. Int J Cardiovasc Sci 30:81–90.
52. Blumer V, Mentz RJ, Sun J-L, et al (2021) Prognostic Role of Prior Heart Failure Hospitalization Among Patients Hospitalized for Worsening Chronic Heart Failure. Circulation: Heart Failure 14:e007871. doi: 10.1161/CIRCHEARTFAILURE.120.007871.
53. Osenenko KM, Kuti E, Deighton AM, et al (2022) Burden of hospitalization for heart failure in the United States: a systematic literature review. J Manag Care Spec Pharm 28:157–167. doi: 10.18553/jmcp.2022.28.2.157.
54. Sawicki KT, Ardehali H (2021) Intravenous Iron Therapy in Heart Failure With Reduced Ejection Fraction: Tackling the Deficiency. Circulation 144:253–255. doi: 10.1161/CIRCULATIONAHA.121.054271.
55. von Haehling S, Ebner N, Evertz R, et al (2019) Iron Deficiency in Heart Failure: An Overview. JACC: Heart Failure 7:36–46. doi: 10.1016/j.jchf.2018.07.015.
56. Tan N, Cai Y, Liu J, et al (2022) Effects and Safety of Oral Iron for Heart Failure with Iron Deficiency: A Systematic Review and Meta-Analysis with Trial Sequential Analysis. Cardiovascular Therapeutics 2022:e6442122. doi: 10.1155/2022/6442122.
57. McDonagh TA, Metra M, Adamo M, et al (2023) 2023 Focused Update of the 2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. European Heart Journal 44:3627–3639. doi: 10.1093/eurheartj/ehad195.
58. Abouzid M, Tanashat M, Khlidj Y, et al (2024) Efficacy and safety of intravenous iron therapy in heart failure patients with iron deficiency: a systematic review and meta-analysis of randomized controlled trials. European Heart Journal 45:ehae666.3278. doi: 10.1093/eurheartj/ehae666.3278.

Supplementary data

Table S1. Search strategy. ("Heart failure" OR "Cardiac Failure" OR " Heart Decompensation" OR "Myocardial Failure" OR "HFrEF" OR "HF" OR "HFpEF") AND ("intravenous iron" OR "ferric carboxymaltose" OR "iron derisomaltose" OR "iron supplementation" OR "iron therapy" OR "iron sucrose" OR "iron isomaltoside" OR "ferric gluconate"). Date/ 31/08/2023

Database	Search terms	Search field	Search results
Pubmed	("Heart failure" OR "Cardiac Failure" OR " Heart Decompensation" OR "Myocardial Failure" OR "HFrEF" OR "HF" OR "HFpEF") AND ("intravenous iron" OR "**ferric** carboxymaltose" OR "iron derisomaltose" OR "iron supplementation" OR "iron therapy" OR "iron sucrose" OR "iron isomaltoside" OR "ferric gluconate")	All Field	598
Cochrane	("Heart failure" OR "Cardiac Failure" OR " Heart Decompensation" OR "Myocardial Failure" OR "HFrEF" OR "HF" OR "HFpEF") AND ("intravenous iron" OR "ferric carboxymaltose" OR "iron derisomaltose" OR "iron supplementation" OR "iron therapy" OR "iron sucrose" OR "iron isomaltoside" OR "ferric gluconate")	Title, Abstract, Keywords	245
WOS	("Heart failure" OR "Cardiac Failure" OR " Heart Decompensation" OR "Myocardial Failure" OR "HFrEF" OR "HF" OR "HFpEF") AND ("intravenous iron" OR "ferric carboxymaltose" OR "iron derisomaltose" OR "iron supplementation" OR "iron therapy" OR "iron sucrose" OR "iron isomaltoside" OR "ferric gluconate")	Abstract	417
SCOPUS	("Heart failure" OR "Cardiac Failure" OR " Heart Decompensation" OR "Myocardial Failure" OR "HFrEF" OR "HF" OR "HFpEF") AND ("intravenous iron" OR "ferric carboxymaltose" OR "iron derisomaltose" OR "iron supplementation" OR "iron therapy" OR "iron sucrose" OR "iron isomaltoside" OR "ferric gluconate")	Abstract	500
EMBASE	Session Results ..... No. Query Results — Results — Date #3. #1 AND #2 — 985 — 31 Aug 2023 #2. 'intravenous iron':ti,ab,kw OR 'ferric carboxymaltose':ti,ab,kw OR 'iron derisomaltose':ti,ab,kw OR 'iron supplementation':ti,ab,kw OR 'iron therapy':ti,ab,kw OR 'iron sucrose':ti,ab,kw OR 'iron isomaltoside':ti,ab,kw OR 'ferric gluconate':ti,ab,kw — 14,830 — 31 Aug 2023 #1. 'cardiac failure':ti,ab,kw OR 'heart failure':ti,ab,kw OR 'myocardial failure':ti,ab,kw OR hfref:ti,ab,kw OR hf:ti,ab,kw OR hfpef:ti,ab,kw — 431,302 — 31 Aug 2023	All Field	985

Figure S1. Subgroup analysis according to chronic or acute heart failure for cardiovascular mortality.

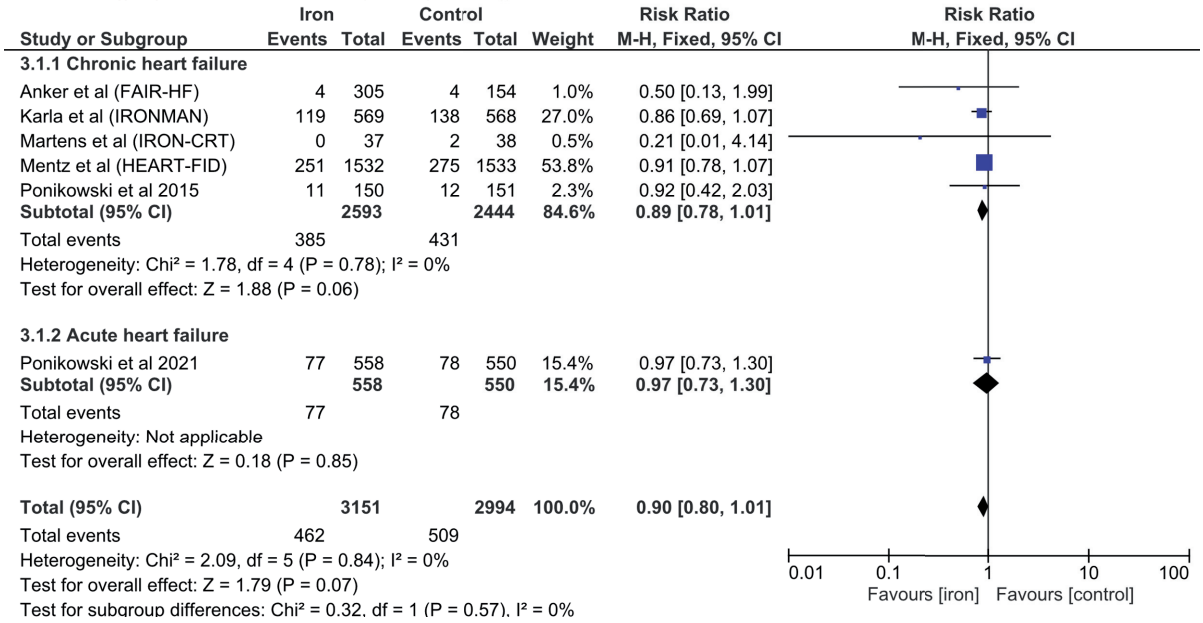


Figure S2. Subgroup analysis according to chronic or acute heart failure for all-cause mortality.

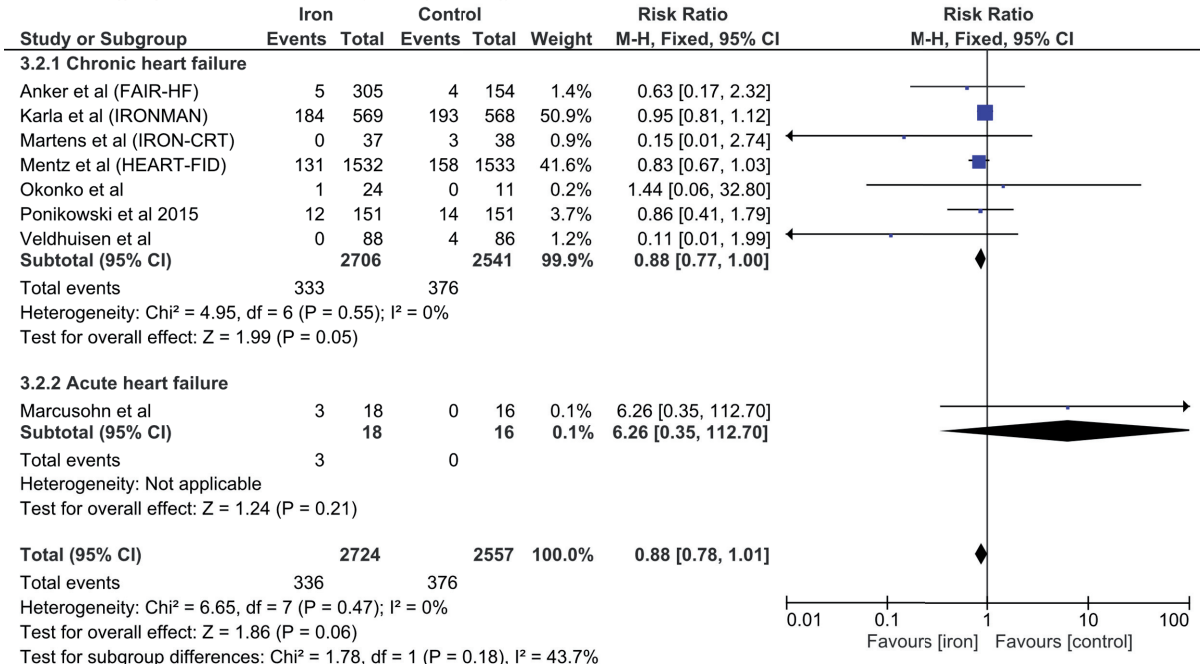


Figure S3. Subgroup analysis according to chronic or acute heart failure for Hospital admission for heart failure (first event).

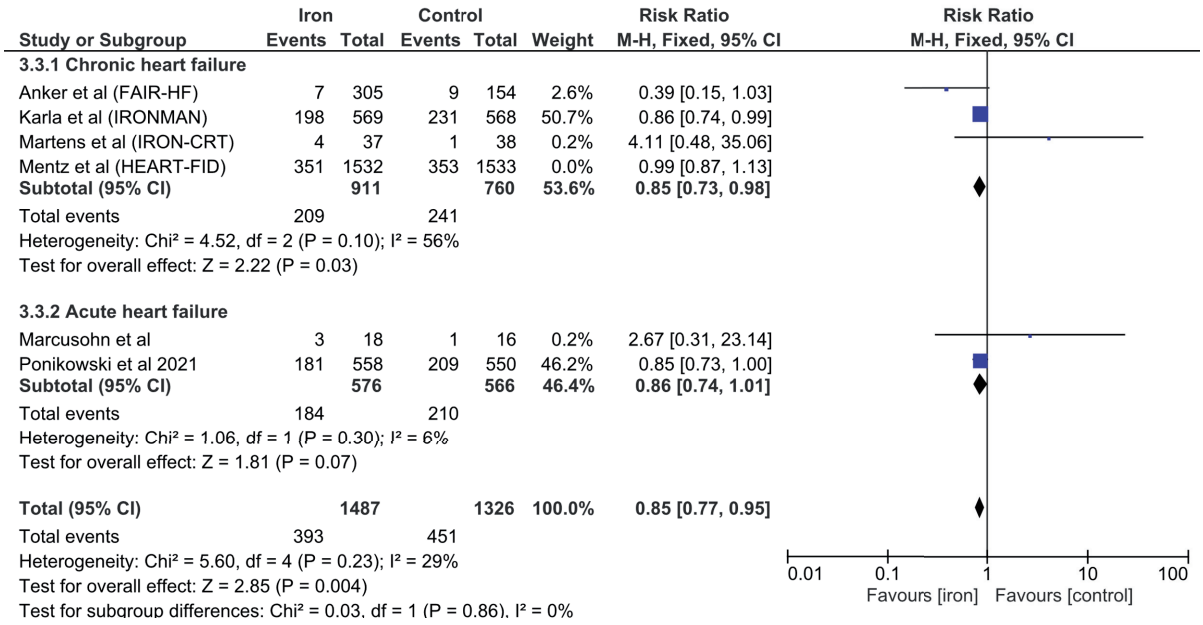


Figure S4. Subgroup analysis according to chronic or acute heart failure for Hospital admission for heart failure (total event).

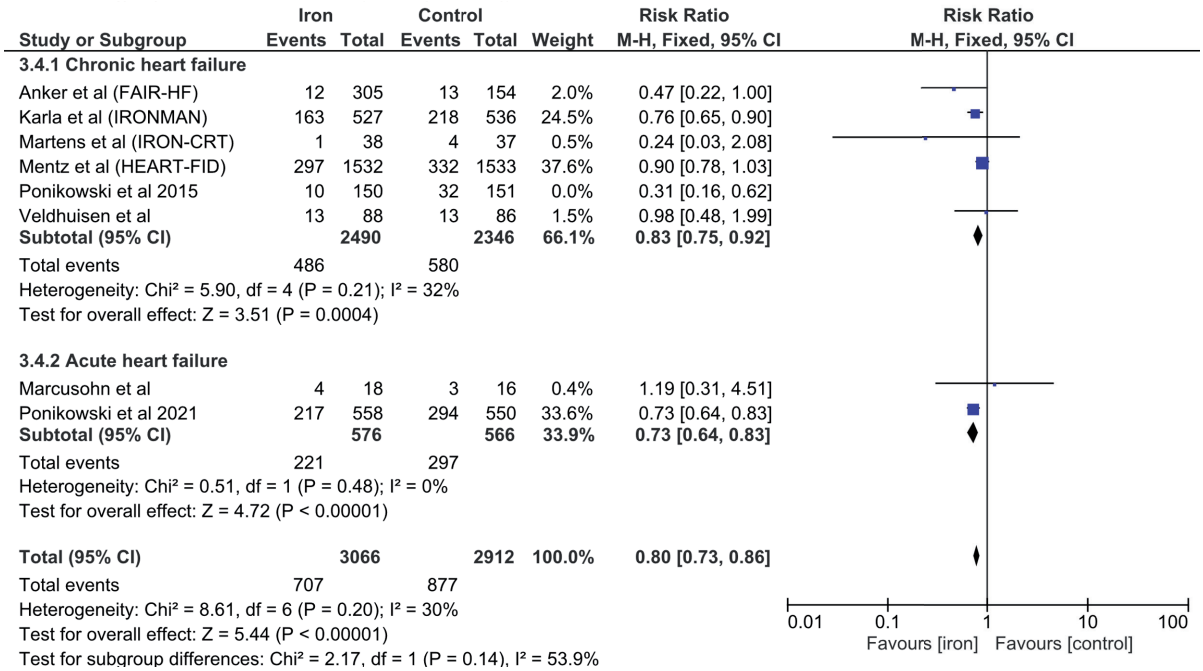


Figure S5. Subgroup analysis according to chronic or acute heart failure for cardiovascular death and hospital admission for heart failure (number of events) rater per 100 patient-year.

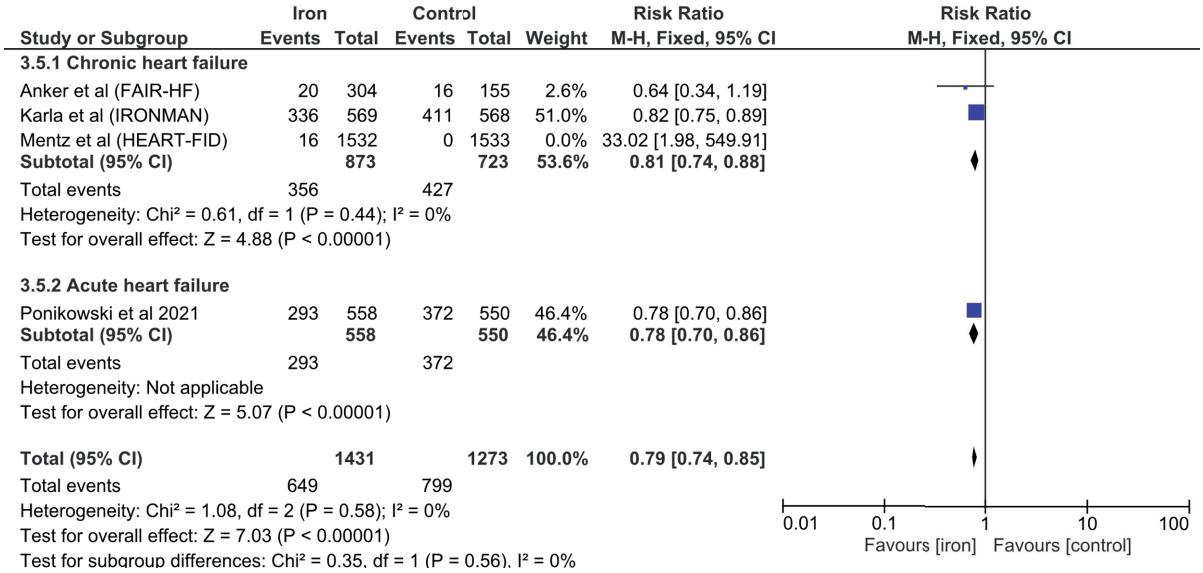


Figure S6. Subgroup analysis according to chronic or acute heart failure for hospital admission for heart failure (number of events) rater per 100 patient-year.

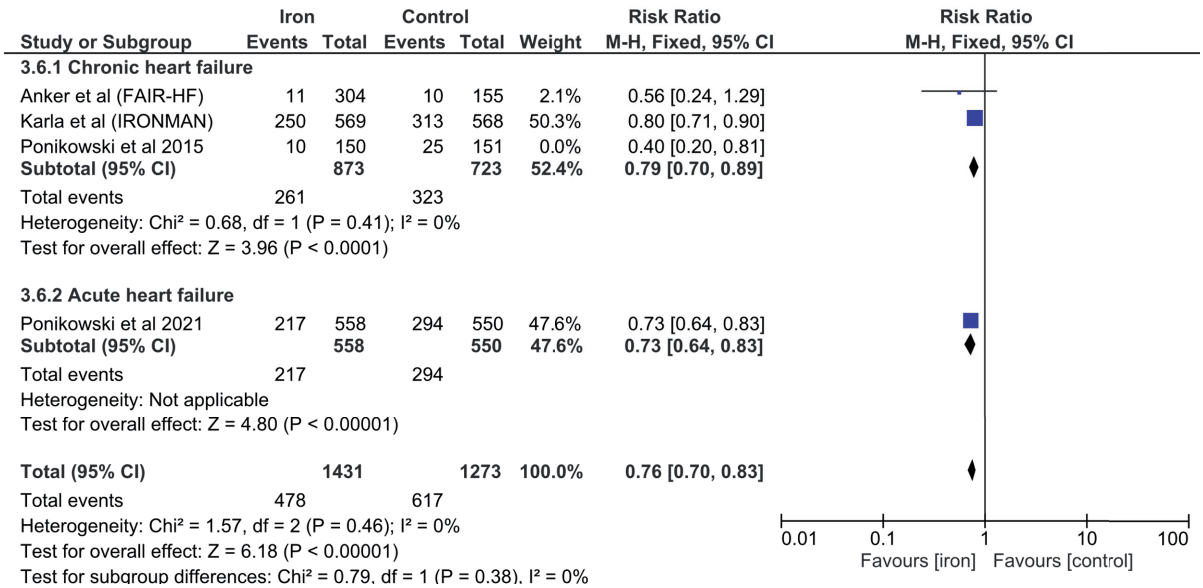


Figure S7. Subgroup analysis according to the iron preparation for cardiovascular mortality.

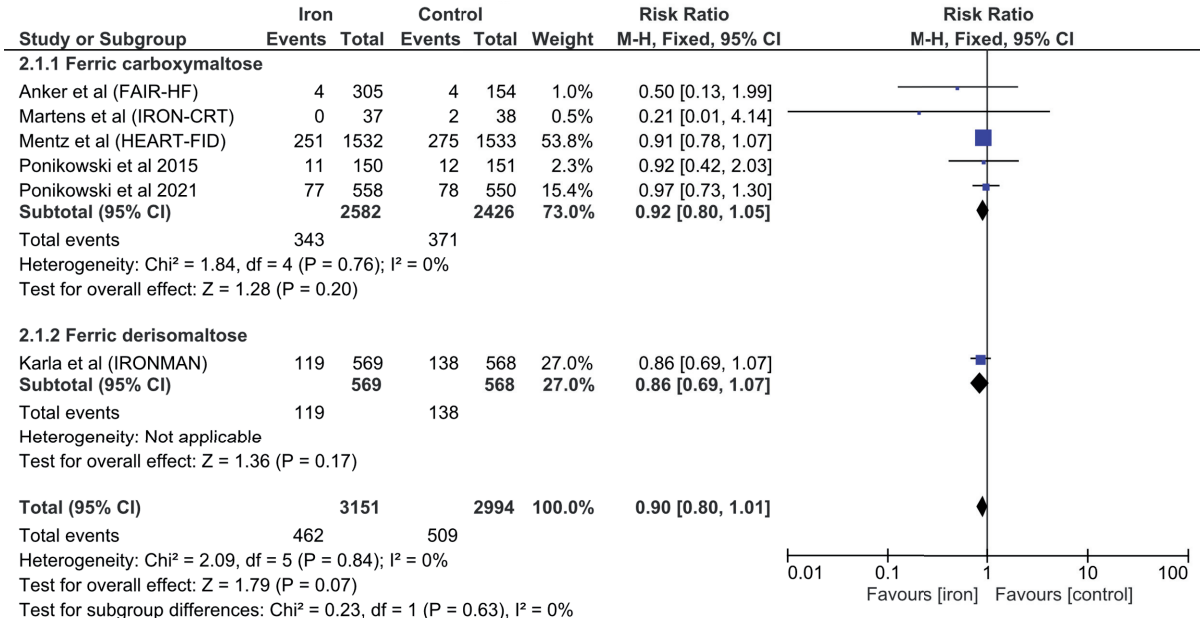


Figure S8. Subgroup analysis according to the iron preparation for all cause mortality.

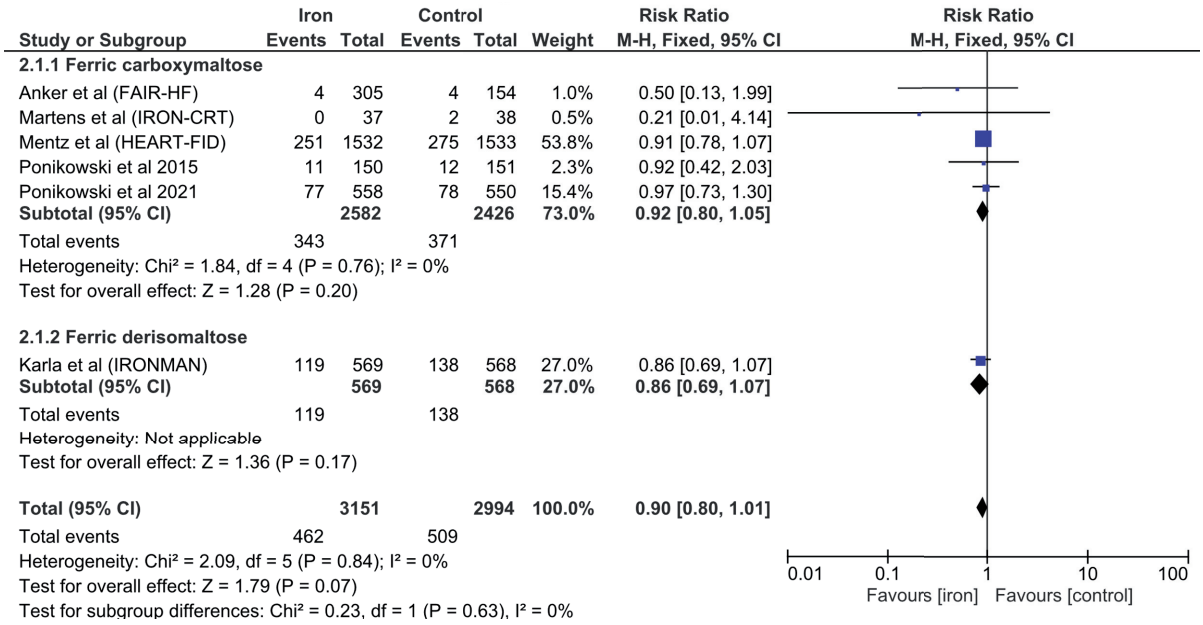


Figure S9. Subgroup analysis according to the iron preparation for hospital admission for heart failure (first event).

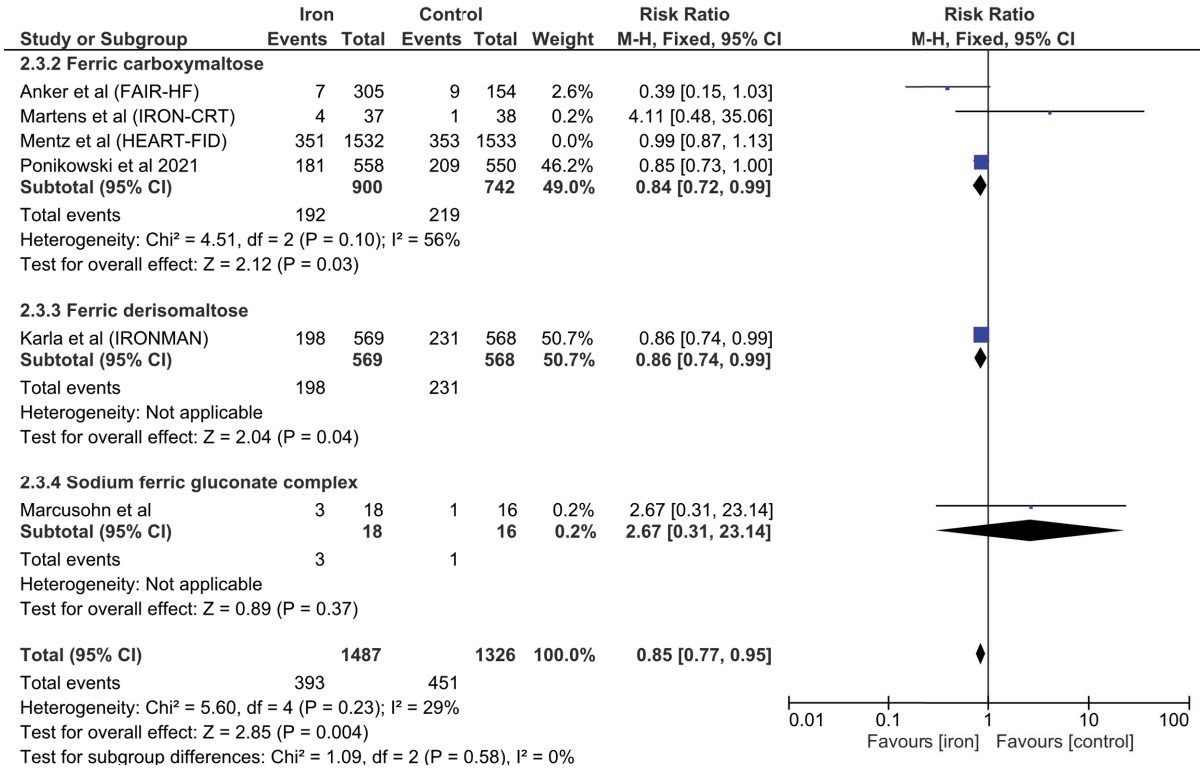


Figure S10. Subgroup analysis according to the iron preparation for hospital admission for heart failure (total event).

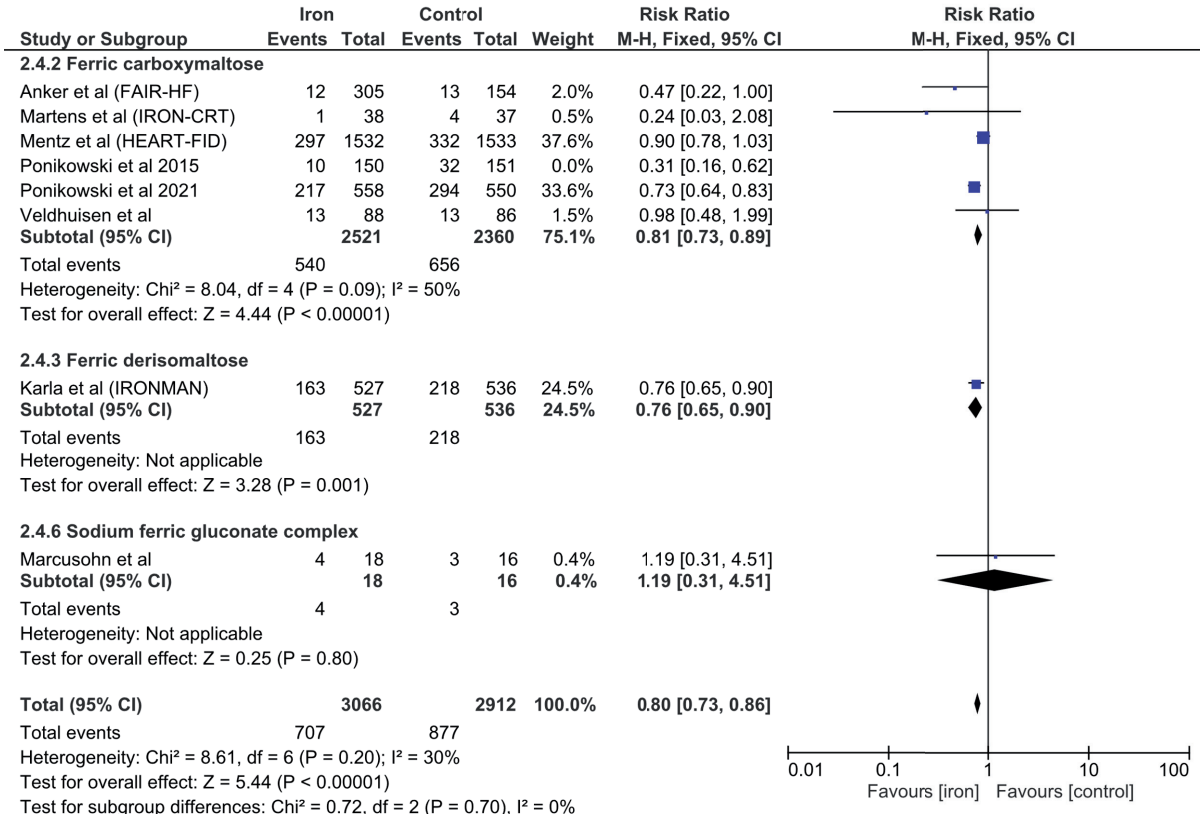


Figure S11. Subgroup analysis according to the iron preparation for cardiovascular death and hospital admission for heart failure (number of events) rater per 100 patient year.

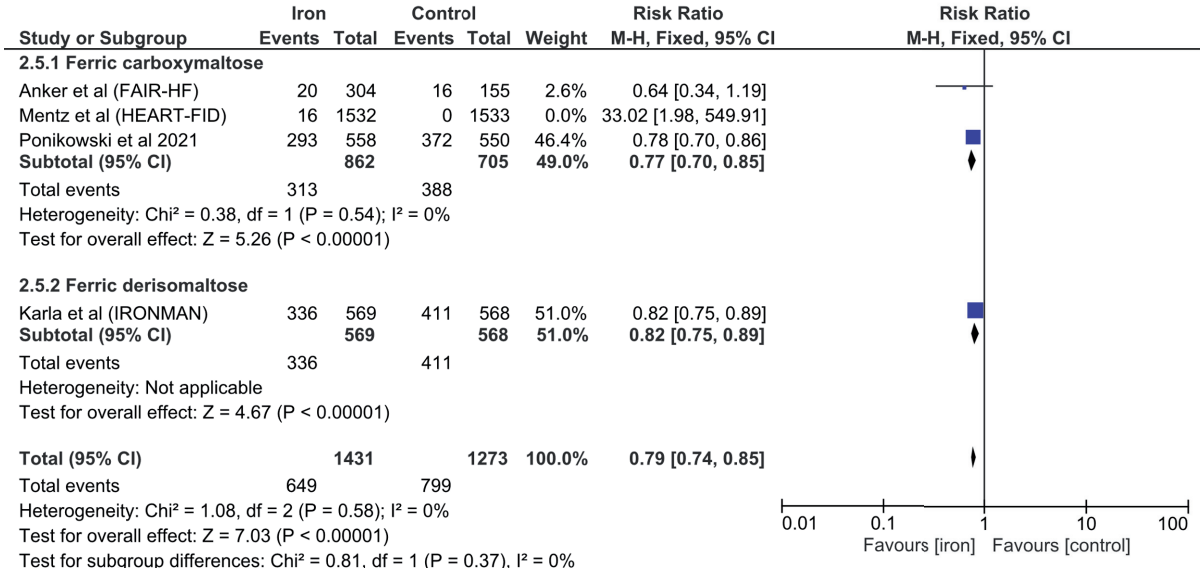


Figure S12. Subgroup analysis according to the iron preparation for hospital admission for heart failure (number of events) rater per 100 patient year.

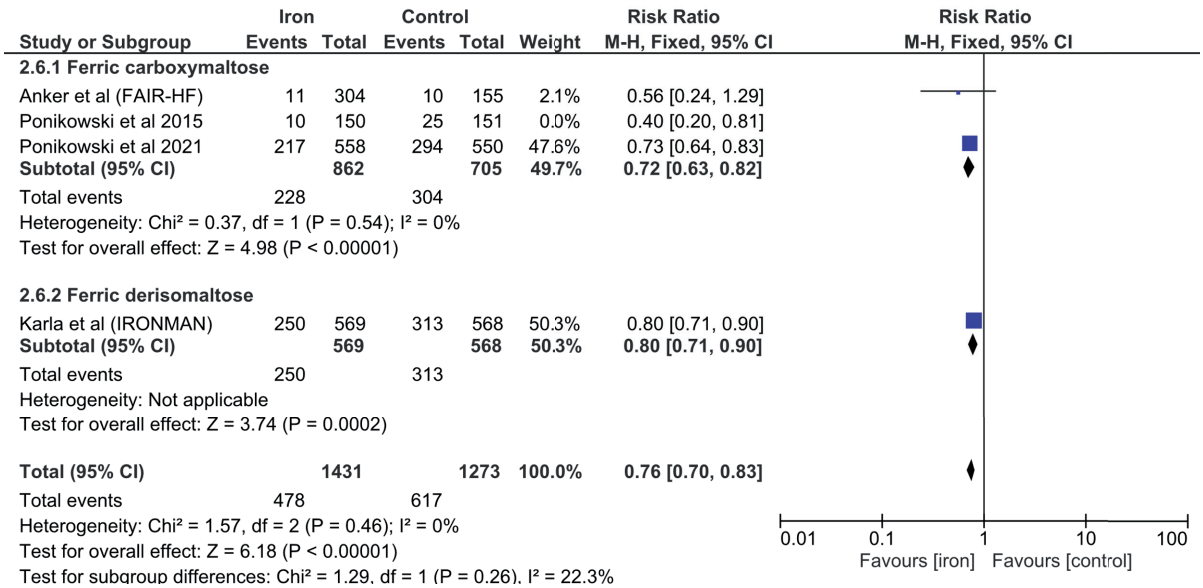


Figure S13. Subgroup analysis according to chronic or acute heart failure for cardiac disorder.

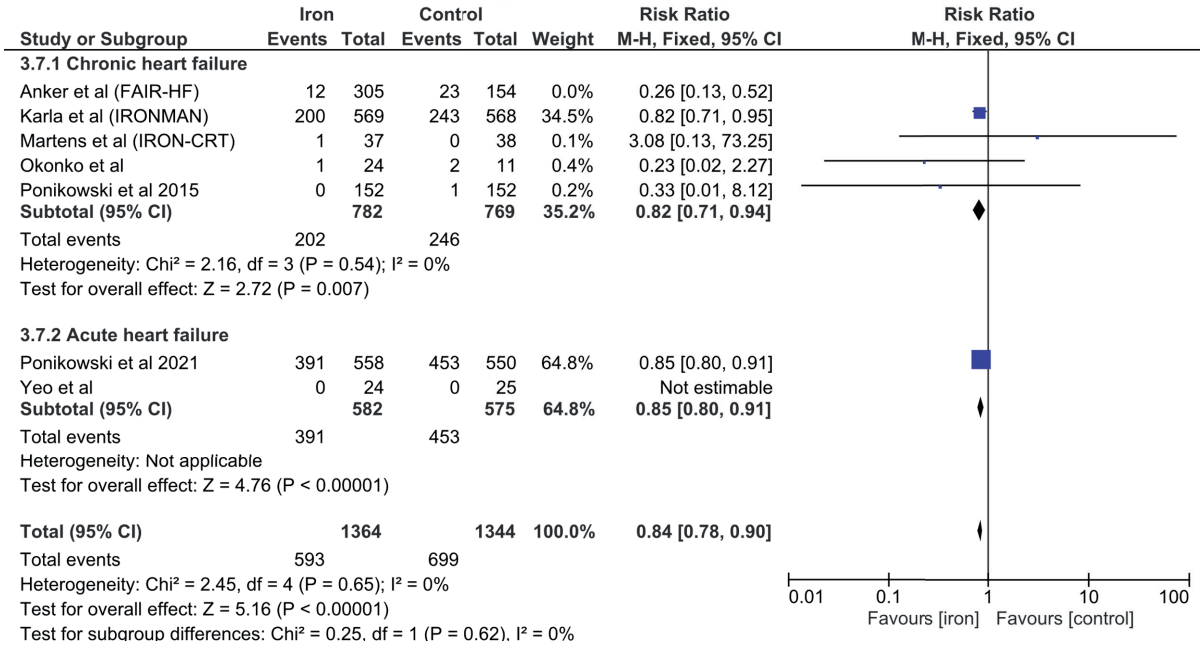


Figure S14. Subgroup analysis according to chronic or acute heart failure for any serious adverse event.

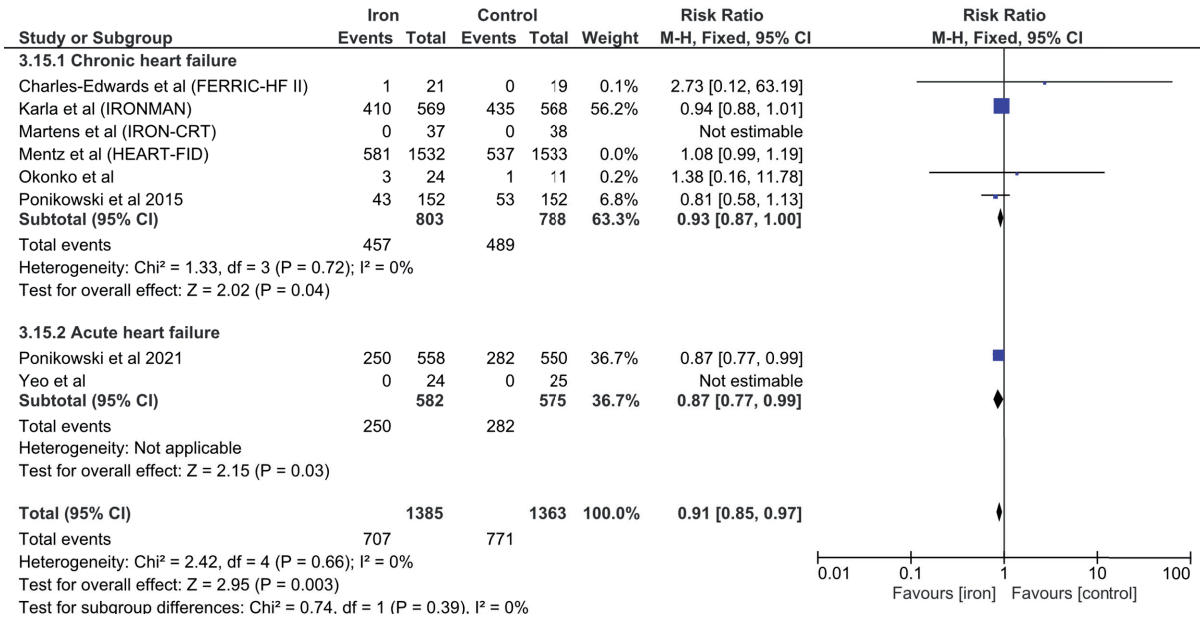


Figure S15. Subgroup analysis according to chronic or acute heart failure for respiratory, thoracic, or mediastinum disorder.

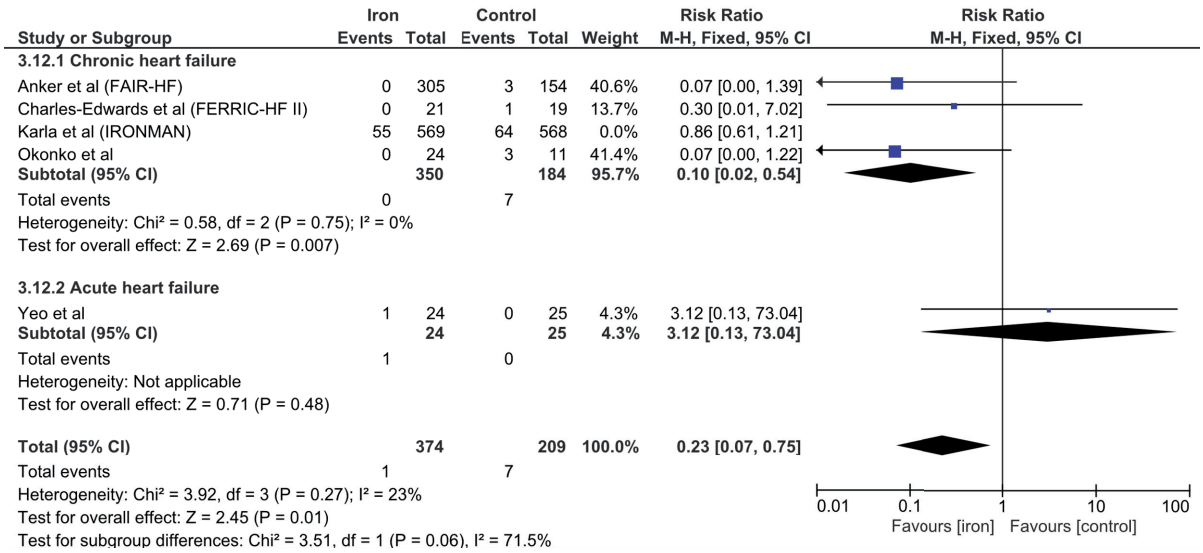


Figure S16. Subgroup analysis according to chronic or acute heart failure for gastrointestinal tract disorder.

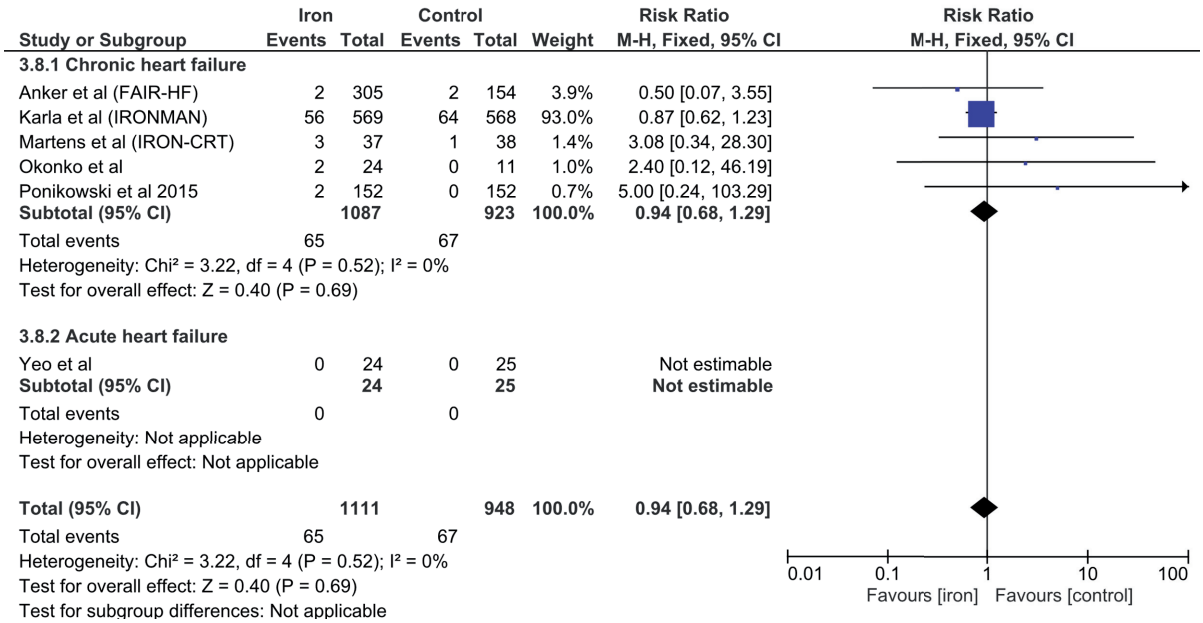


Figure S17. Subgroup analysis according to chronic or acute heart failure for nerves system disorder.

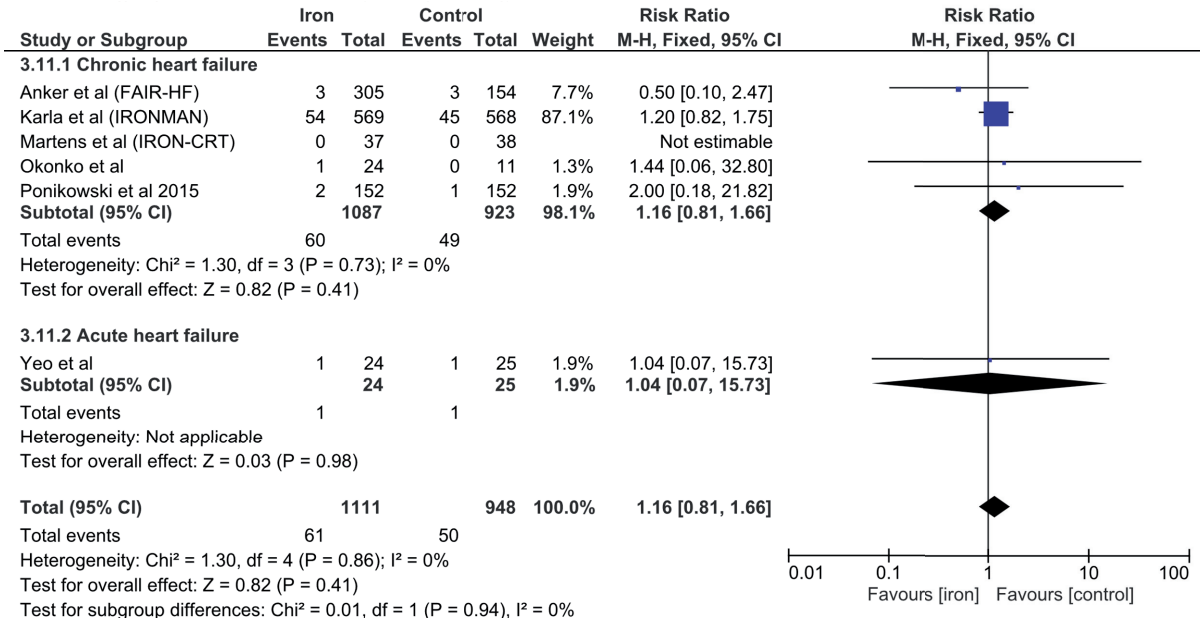


Figure S18. Subgroup analysis according to chronic or acute heart failure for vascular disorder.

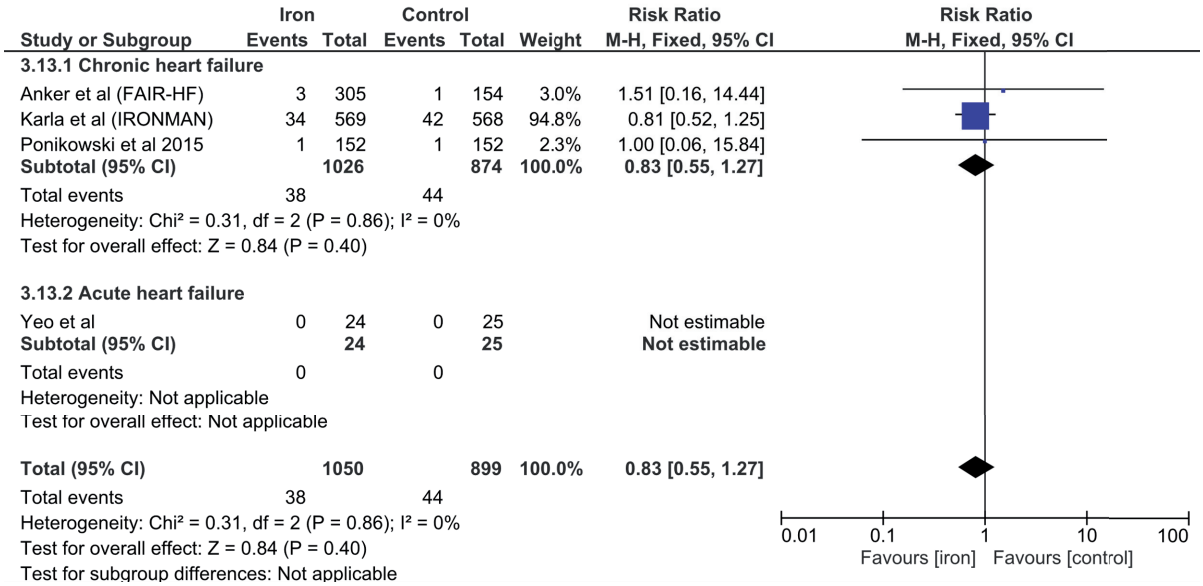


Figure S19. Subgroup analysis according to the iron preparation for cardiac disorder

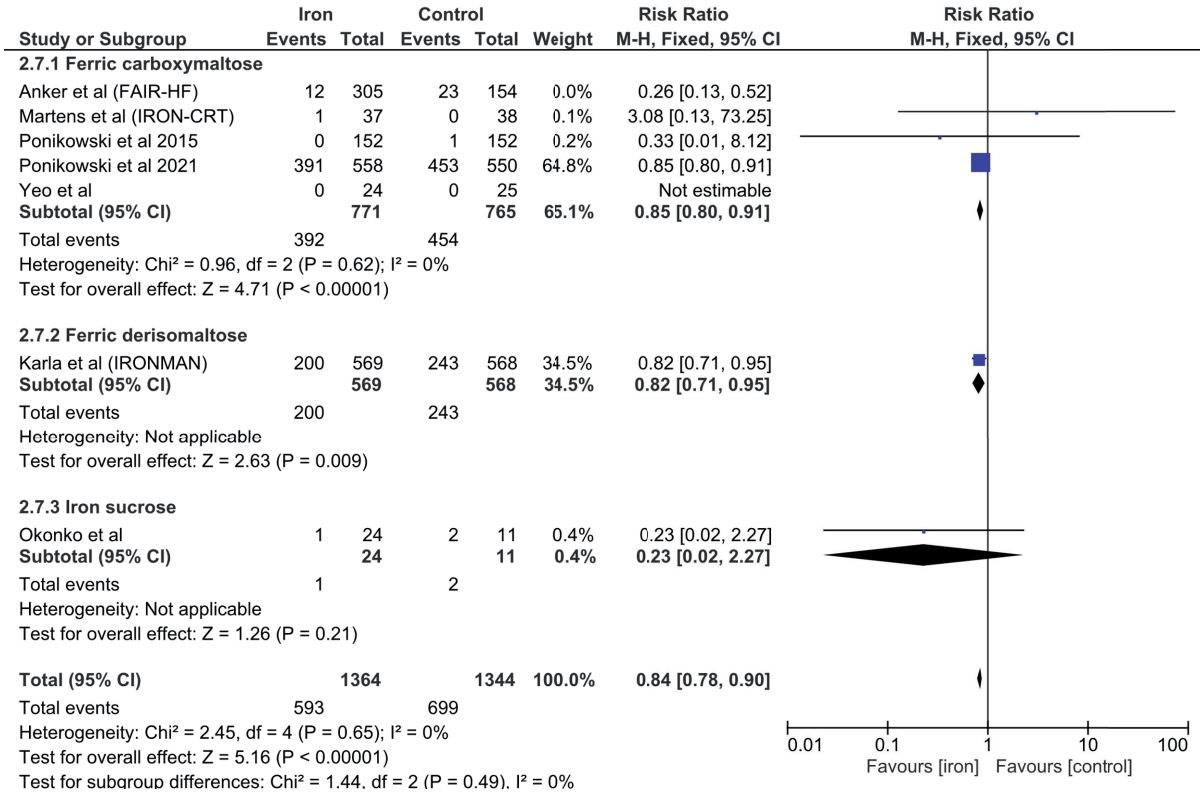


Figure S20. Subgroup analysis according to the iron preparation for any serious adverse effect.

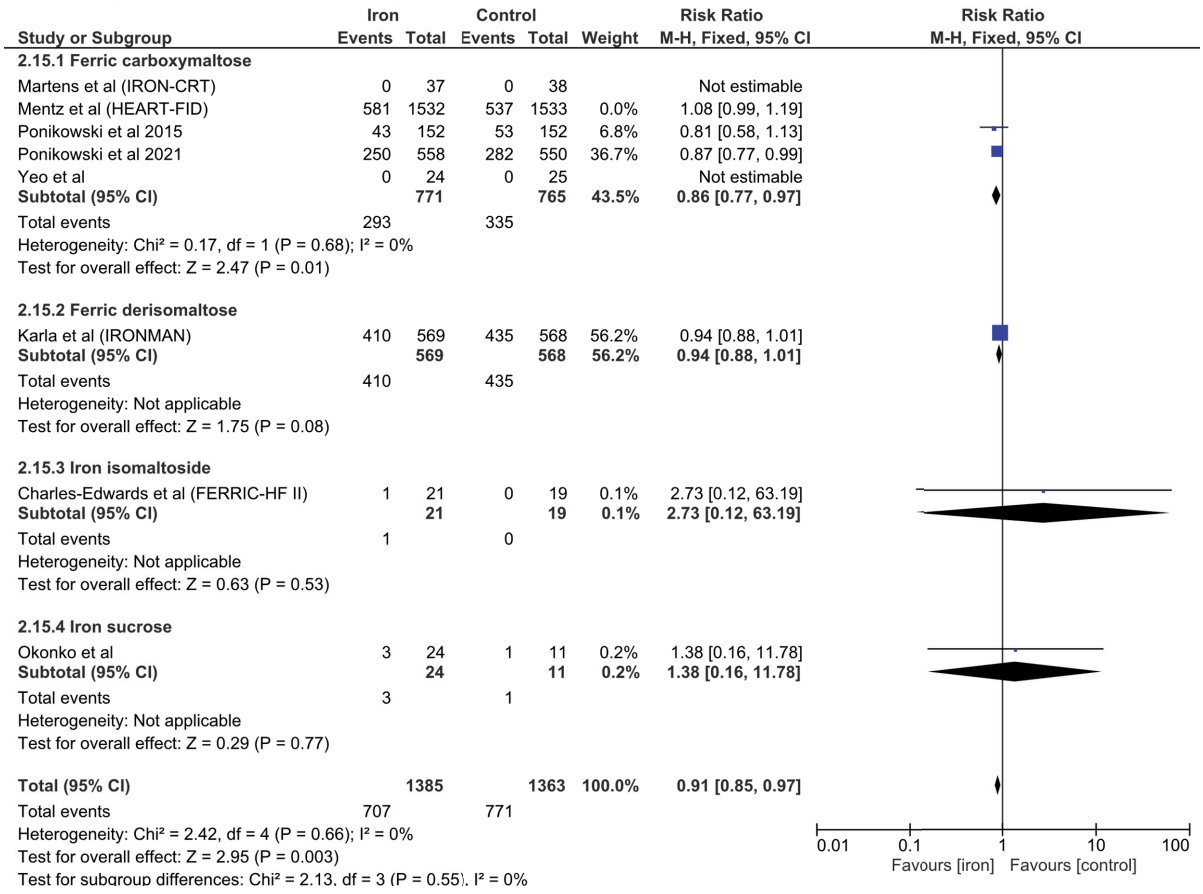


Figure S21. Subgroup analysis according to the iron preparation for any gastrointestinal tract disorder.

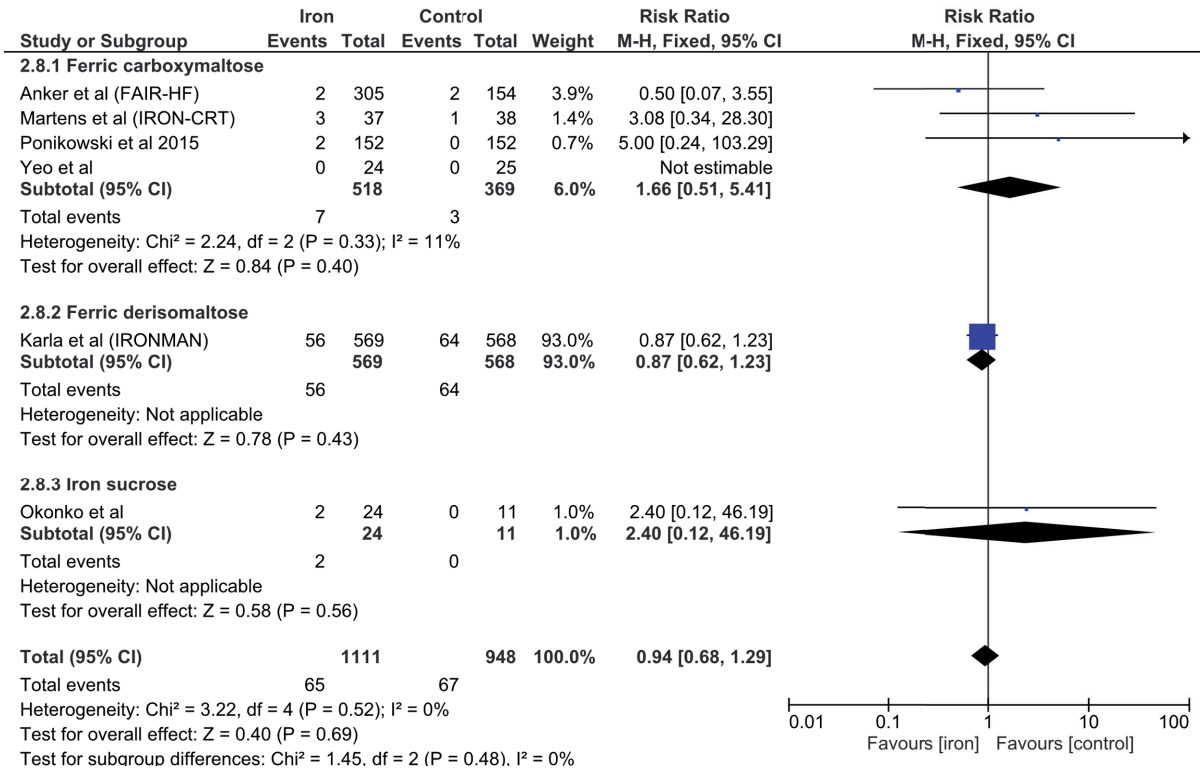


Figure S22. Subgroup analysis according to the iron preparation for injection site condition

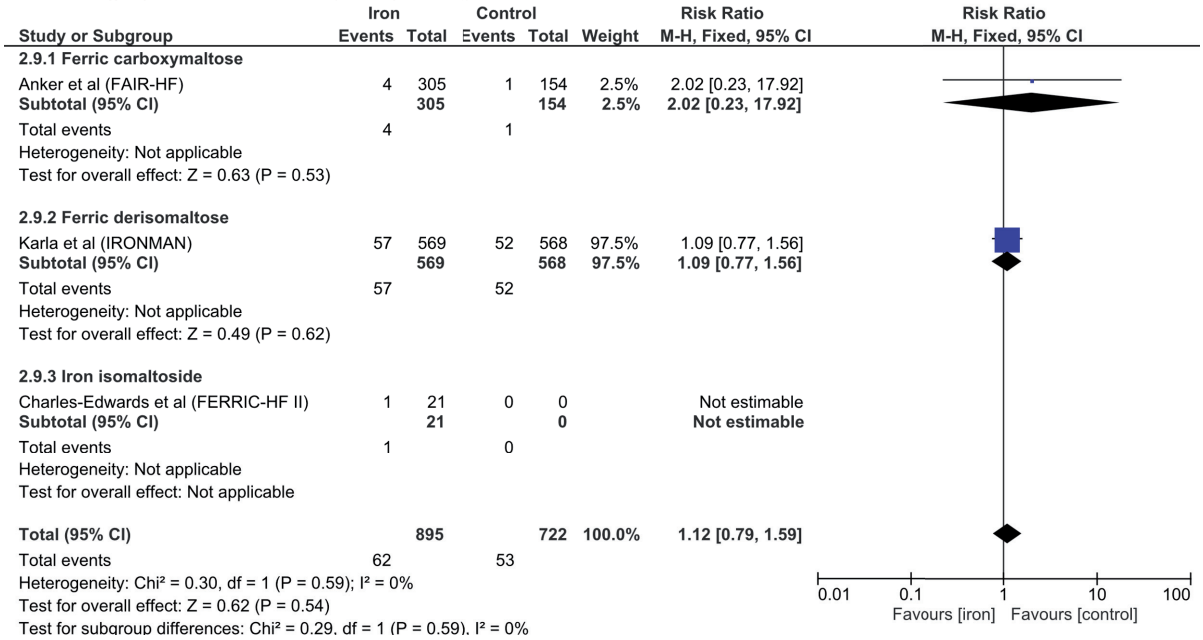


Figure S23. Subgroup analysis according to the iron preparation for infection.

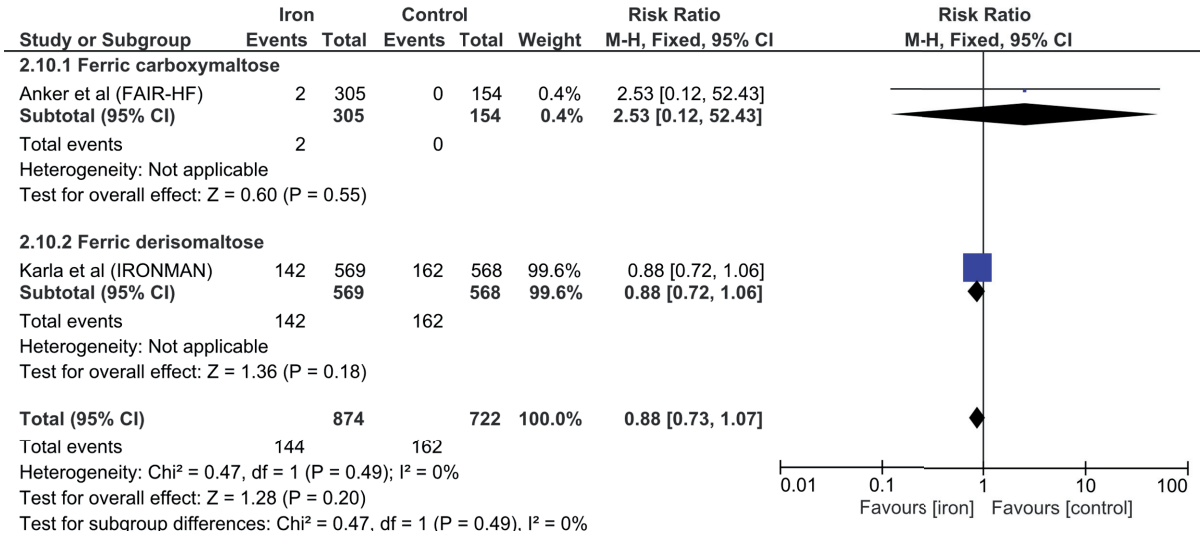


Figure S24. Subgroup analysis according to the iron preparation for nerves system disorder.

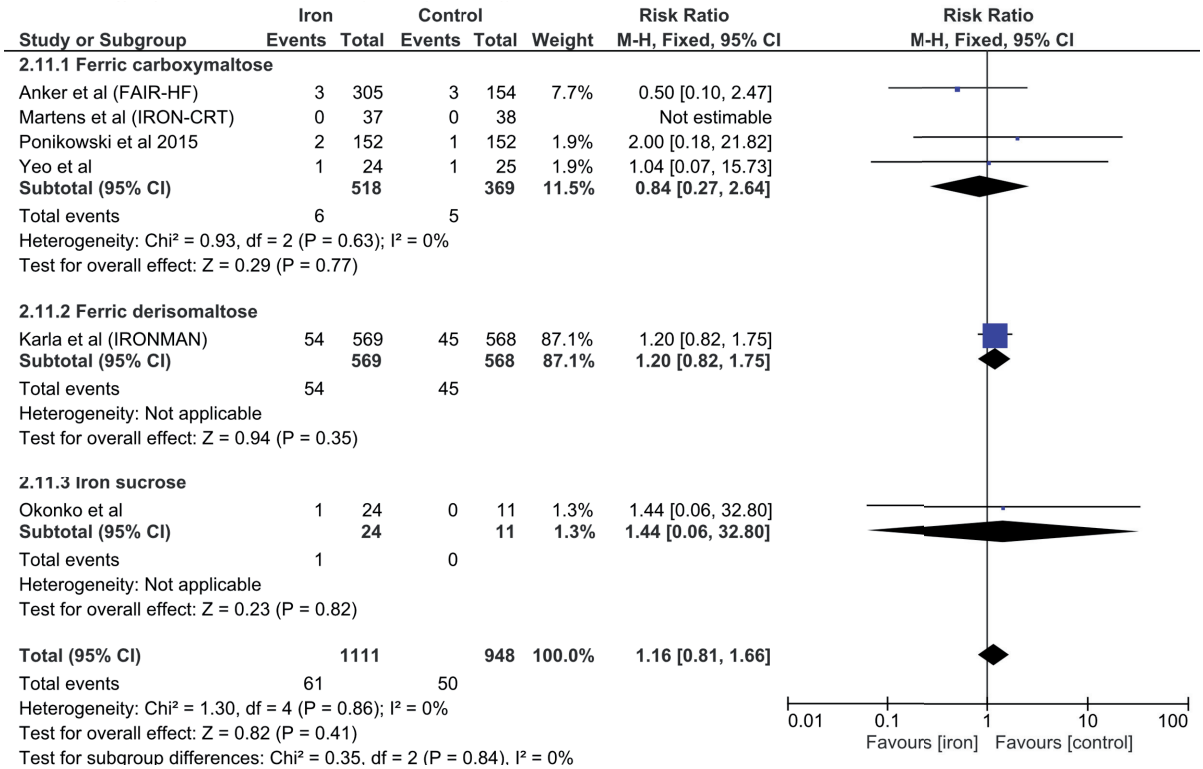


Figure S25. Subgroup analysis according to the iron preparation for respiratory, thoracic or mediastinum disorder.

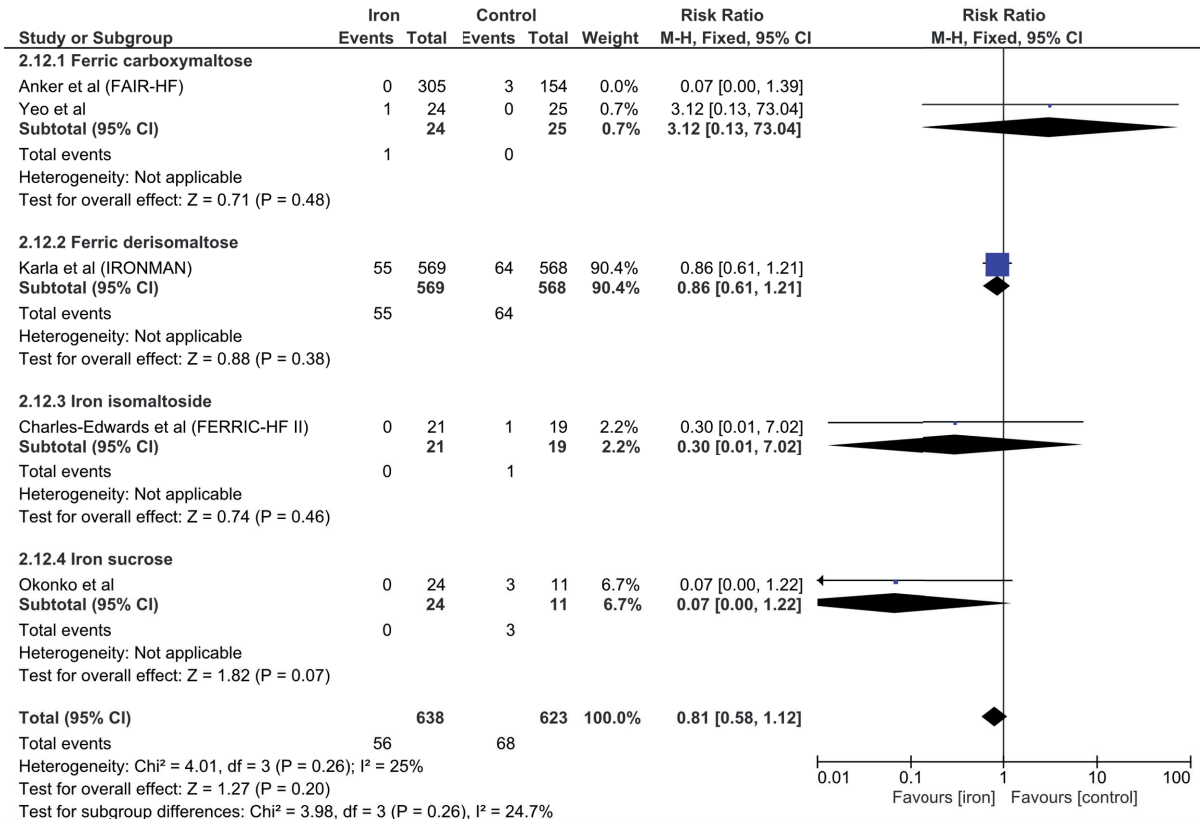


Figure S26. Subgroup analysis according to the iron preparation for vascular disorder.

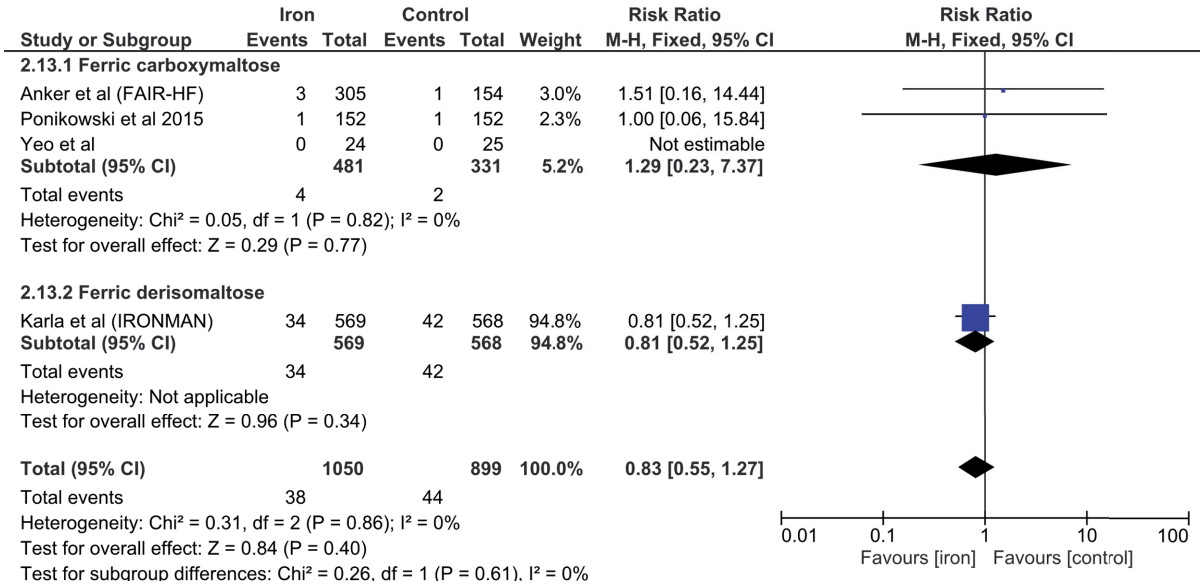


Figure S27. Subgroup analysis according to the iron preparation for any adverse effect.

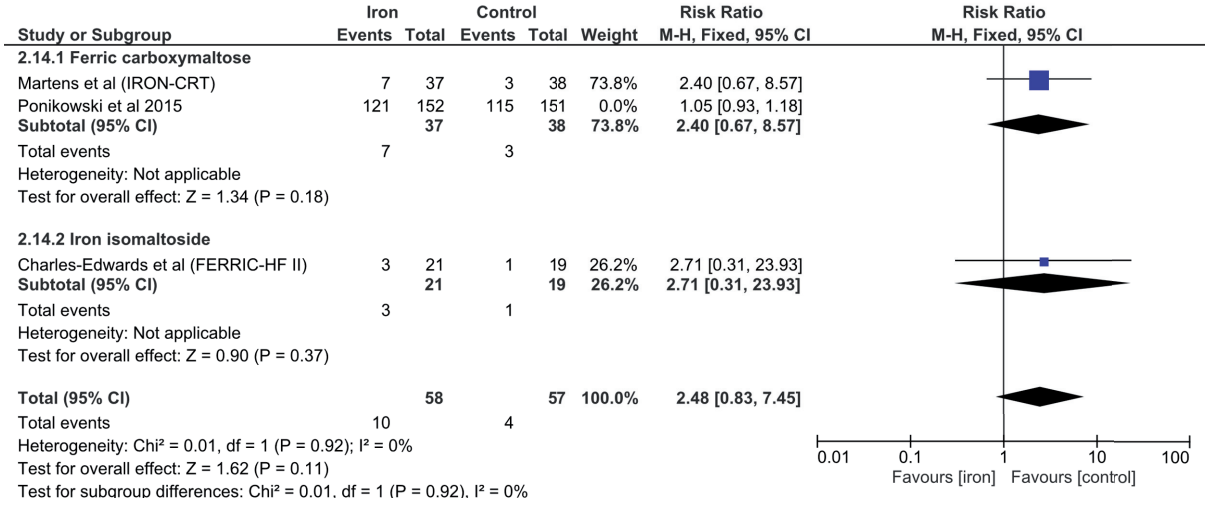


Figure S28. Subgroup analysis according to the iron preparation for any adverse event leading to withdrawal.

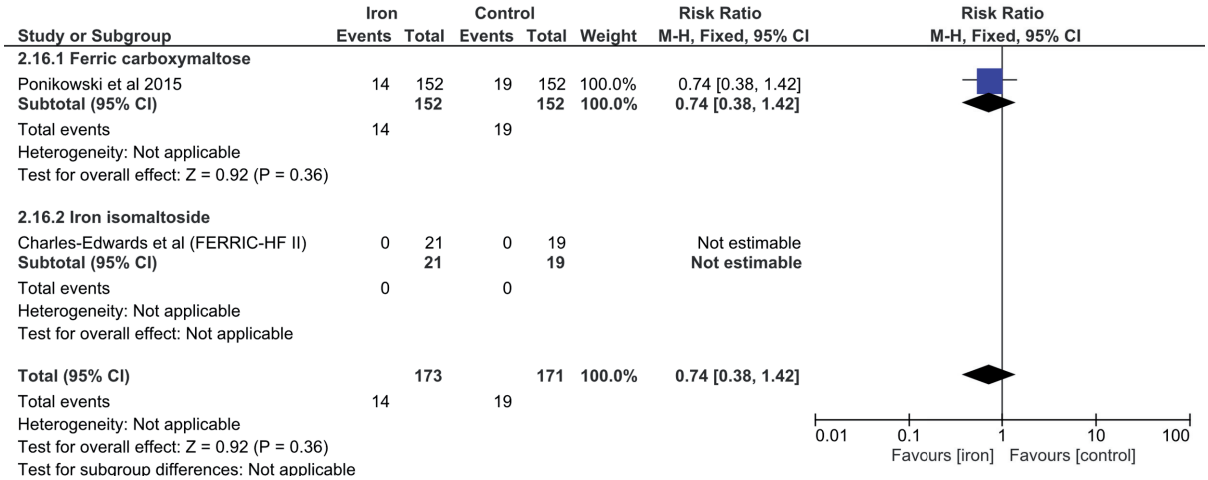
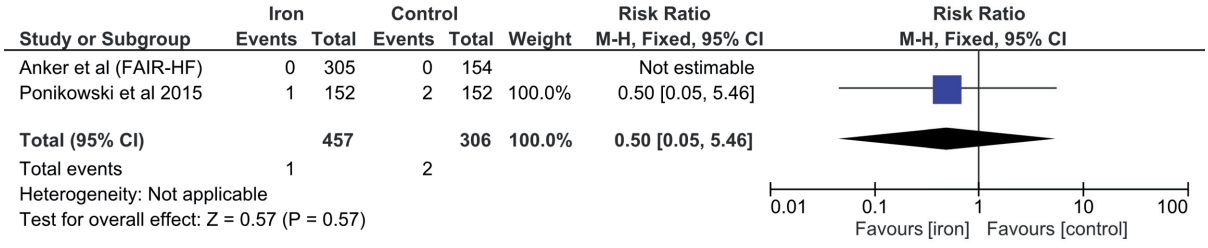


Figure S29. Subgroup analysis according to the iron preparation for abnormal lab test, vital sign or physical finding



JMS Journal of Medical Science

The effect of low advanced glycation end product diet on bone health and cardio-metabolic parameters in overweight and obese postmenopausal women: study protocol for a randomised controlled trial (AGEs study)

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ABSTRACT

Advanced glycation end products (AGEs) have been suggested to play an important role in osteoporosis. However, no randomised controlled trial has investigated the effect of a diet with low AGEs on densitometric parameters. This study will evaluate the effect of a 12-month low AGEs diet on bone health and cardio-metabolic parameters in postmenopausal overweight and obese women. In total, 80 subjects will be included in the study and randomly divided into two groups: low AGEs diet and standard AGEs diet for 12 months. The mineral density and content will be measured at the lumbar spine, femoral neck, and total body during the pre- and post-intervention period. Selected markers of bone formation and resorption will be assessed. Anthropometric parameters and body composition will be evaluated and markers of glucose, insulin, lipid metabolism, inflammatory and endothelial parameters will be measured. Adherence to the diet will be monitored using dietary records.

Research project objectives

The study's primary aim will be to assess the effect of low advanced glycation end products (AGEs) diet on bone mineral density (BMD) and content (BMC), as well as markers of bone formation and resorption in overweight and obese postmenopausal women. Moreover, the study will examine the impact of a low AGEs diet on cardio-metabolic parameters, including anthropometric parameters, body composition, glucose and insulin homeostasis, lipid metabolism, endothelial dysfunction parameters and inflammatory markers. The following research null hypotheses were formulated:

1. Low AGEs diet does not affect BMD and BMC in overweight and obese postmenopausal women.
2. Low AGEs diet does not affect markers of bone formation and resorption in overweight and obese postmenopausal women.
3. Low AGEs diet does not affect anthropometric parameters and body composition in overweight and obese postmenopausal women.
4. Low AGEs diet does not affect glucose and insulin homeostasis in overweight and obese postmenopausal women.
5. Low AGEs diet does not affect lipid metabolism in overweight and obese postmenopausal women.
6. Low AGEs diet does not affect endothelial dysfunction parameters in overweight and obese postmenopausal women.
7. Low AGEs diet does not affect inflammatory parameters in overweight and obese postmenopausal women.
8. Low AGEs diet does not affect blood pressure in overweight and obese postmenopausal women.

Research plan and basic concept

Basic concept

The menopause period is often a turning point in a woman's life. Menopause is associated with an increase in fat mass, insulin resistance, dyslipidaemia and endothelial dysfunction, as well as a higher prevalence of osteoporosis [1]. Decreased ovarian oestrogen production and relative androgen excess around menopause onset are some of the most studied factors linking menopause, bone and cardiometabolic health [2]. Excessive body weight, often observed in postmenopausal women, seems to have a particularly unfavourable effect on bone metabolism and cardio-metabolic markers. Unfortunately, factors protecting against the development of perimenopausal disorders have not been identified so far [3].

AGEs include a diverse group of compounds that are the products of nonenzymatic reactions between reducing sugars and proteins, lipids or nucleic acids [4]. The majority of AGEs are obtained from the consumption of food. Foods of animal origin, particularly those rich in protein and fat, exhibit the highest levels of AGEs, whereas carbohydrate-rich foods demonstrate the lowest amounts. Additionally, cooking methods such as grilling, roasting, broiling, or frying produce higher levels of AGEs than boiling, poaching, stewing, or steaming [5].

AGEs have a significant impact on human health. Previous studies showed an association between AGEs levels and a variety of conditions such as diabetes mellitus [6], cardiovascular diseases [7], metabolic syndrome [8], Alzheimer's disease [9], some cancer [10] or polycystic ovary syndrome [11]. Elevated AGEs levels were also associated with a higher risk of all-cause mortality [12]. Several studies also investigated the effects of AGEs on bone health [13–17]. It has been suggested that AGEs might affect bone through their accumulation in collagen fibres [13]. Moreover, AGEs have been found to significantly inhibit osteoblast proliferation, differentiation and mineralisation and induce osteoblast apoptosis [14]. Circulating AGEs also lead to decreased bone strength by damage to structural bone [15]. Higher AGEs levels were observed in subjects with osteoporosis and osteopenia compared to healthy participants. Moreover, a negative correlation was found between AGEs levels and BMD. Yang et al. [16] demonstrated that women with elevated AGEs concentrations had a 5.34 times higher risk of developing osteopenia in terms of the lumbar spine T-score and a 3.31 times higher risk of osteopenia in relation to the hip T-score. In another study, pentosidine, a type of AGEs, was negatively correlated with BMC in adolescents, suggesting that the accumulation of AGEs may affect peak bone mass in young people [17]. While numerous studies have shown the negative impact of a high AGEs diet on bone properties in animal models [18–19], research is scarce regarding the role of dietary AGEs in human bone health. Only one human study investigated the relationship between AGEs intake and bone health. It demonstrated a positive association with the prevalence of prevalent vertebral, a non-significant trend for major osteoporotic fractures, and no association with BMD and trabecular bone score [20].

Several studies demonstrated a relationship between dietary AGEs intake and cardio-metabolic parameters [21–25]. A recent meta-analysis reported a reduction in insulin resistance, fasting insulin, total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) in the low AGEs group compared with the high AGEs group [21]. Moreover, another meta-analysis has shown a significant decrease in body mass index (BMI), weight and leptin and an increase in adiponectin levels after consumption of the low AGEs diet compared to the high AGEs diet [22]. On the other hand, other studies showed that consumption of low AGEs diet did not improve the inflammatory and endothelial markers [23–25]. Nevertheless, it has been reported that the reduction in dietary AGEs with a low-fat plant-based diet was associated with a significant reduction in the frequency of severe and moderate-to-severe postmenopausal hot flashes, independent of changes in energy intake and weight loss [26]. However, most trials investigating the effect of a low AGEs diet are limited by a short duration period and a small sample size. Therefore, further randomised controlled trials with a correct methodology and increased quality assessing the effect of low AGEs diet on cardio-metabolic markers are needed.

Research plan

The study followed a parallel-group, prospective, randomised controlled trial design. The protocol was registered in the Deutsches Register Klinischer Studien (registration number: DRKS00034643, registration date: 16 July 2024) and was developed in accordance with the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) guidelines [27 28]. Ethical approval for the study was obtained from the Bioethical Committee of Poznań University of Medical Sciences (ref. 112/24, dated 8 February 2024), and the research will be conducted in compliance with the principles of the Declaration of Helsinki [29]. Funding for the study was provided by the Nutricia Foundation (Bobrowiecka 8 Str., Warsaw, Poland). However, the sponsor had no involvement in the study design and will have no role in data collection, management, analysis, interpretation, manuscript writing, or the decision to submit the paper for publication.

Research team members will obtain informed consent from all study participants. Participants will be informed that they can withdraw from the study at any time without facing any consequences. The informational materials provided to potential volunteers include detailed information about the study's objectives and nature. All biological samples (e.g., blood samples) obtained from participants will be anonymised and identified using unique patient codes. Study participants will be assured that their personal data will

Figure 1. The SPIRIT study schedule of enrolment, interventions and assessments.

	STUDY PERIOD					
	Enrolment	**Allocation**	**Post-allocation**			**Close-out**
TIMEPOINT	$-t_1$	**0**	t_1 (3rd month)	t_2 (6th month)	t_3 (9th month)	t_4 (12th month)
ENROLMENT:						
Eligibility screen	x					
Informed consent	x					
Medical history questionnaire	x					
Hormones levels (FSH, FH, oestradiol, progesterone, PTH)	x					
Allocation		x				
INTERVENTIONS:						
Low AGEs diet		♦—	—	—	—	—♦
Standard AGEs diet		♦—	—	—	—	—♦
ASSESSMENTS:						
Primary outcomes:						
Densitometric parameters (BMC, BMD at the lumbar spine, femoral neck and total body)		x				x
Markers of bone formation and resorption (osteocalcin, BSAP and CTX-I)		x				x
Secondary outcomes:						
Anthropometric parameters (body high, body weight*, waist and hip circumferences, BMI, WHR)	x					x
Biochemical parameters (fasting glucose and insulin levels, lipid profile, inflammatory markers, markers endothelial dysfunction)		x				x
Blood pressure		x				x
Body composition (DEXA)		x				x
***Others*:**						
Food Frequency Questionnaire		x		x		x
International Physical Activity Questionnaire		x		x		x
Socioeconomic assessment		x				x
Three-day dietary record*		x	x	x	x	x

* Assessed every month

BMI – body mass index; BSAP – bone-specific alkaline phosphatase; CTX-I – cross-linked C-terminal telopeptide of type I collagen; DEXA – dual-energy X-ray absorptiometry; FSH – follicle-stimulating hormone; LH –luteinizing hormone; PTH – parathyroid hormone; WHR – waist-to-hip ratio

not be shared with others and that they retain the right to access, correct, or delete their data. Additionally, participants may submit a complaint to the Personal Data Protection Office in Warsaw (Stawki 2 Str., Warsaw, Poland) if they believe their data has been processed in violation of data protection regulations. The study will utilise the RedCap (Vanderbilt University, Nashville, Tennessee, USA) tool for data collection, anonymisation, validation, quality control, and export. Data storage procedures will comply with the regulations of the Poznan University of Medical Sciences, Poznań, Poland. All electronic data will be encrypted and protected against unauthorised access. Anonymised data stored in electronic files will follow strict security measures. For any data stored in paper format, documents will be organised according to participant codes and kept in binders. These binders will be stored in locked cabinets within secure rooms to prevent unauthorised access. Before statistical analysis, all collected data will undergo careful review to identify and correct any errors or anomalies. The final trial dataset will be retained by the Principal Investigator and made available upon reasonable request.

Recruitment for the study will be carried out in clinics, primary healthcare facilities, and other medical centres in Poznań (Poland) and the surrounding areas. Additionally, information about the recruitment will be sent by post to offices, companies, universities, and other educational institutions within the Poznań district, as well as to senior clubs and local media outlets. The recruitment campaign will also be promoted through social media platforms.

Potential participants will be screened by a physician at the Department of Pediatric Gastroenterology and Metabolic Diseases, Poznań University of Medical Sciences, Poland, to ensure compliance with the protocol requirements. The following inclusion criteria will be used:

- Sex: women,
- BMI ≥ 25.0 kg/m^2,
- Age: 50–70 years,
- Menopause: at least 12 months prior to study enrolment.

Exclusion criteria will include:

- Menopause before the age of 40 years,
- Previously diagnosed osteoporosis or other serious bone diseases,
- Taking medications that affect bone metabolism,
- Taking hormone replacement therapy,
- Taking calcium or vitamin K supplements in the last 3 months,
- Diagnosed acute or chronic autoimmune diseases, inflammatory diseases, infectious diseases, viral, bacterial or parasitic infections,
- Malignant neoplastic disease treated with chemo- or radiotherapy within the last 5 years,
- Acute and chronic kidney and liver diseases,
- Underactive or overactive parathyroid glands.

The study population (n = 80) will be randomised (allocation ratio 1:1) into two groups: low AGEs (n = 40) and standard AGEs (n = 40). During the one-year intervention period, participants in the low AGEs group will receive guidance from dietitians on reducing AGE intake. Dieticians will monitor compliance through dietary records and monthly phone calls with the study participants. Based on results from previous studies [22,23], it is estimated that the average AGEs intake in the study population before the intervention will exceed 15,000 kU AGEs/day. During the intervention, the AGEs intake in the low AGEs group is expected to be three times lower than in the standard AGEs group. All participants will be instructed to follow an isocaloric diet and supplement with vitamin D throughout the intervention. They will also be advised to maintain their usual level of physical activity. Additionally, participants will be required to report any adverse effects to the research team.

The minimum sample size was calculated using G*Power software (University of Kiel, Kiel, Germany). To obtain a power of 90% ($\alpha = 0.05$, $\beta = 0.1$), at least 33 subjects per group should be included in the study. Assuming a maximum 20% drop-out rate, at least 40 subjects per group will be recruited.

Randomisation will be performed by Python using numpy library by an independent researcher. Blocked randomisation (with stratification according to age, 25(OH)D levels and whether participants have previously taken vitamin D supplementation) will be performed. A randomisation list will be generated, and the allocation sequence list will be concealed until the interventions are assigned. Neither the study participants nor the research staff will know the allocation

sequences. Due to the nature of the intervention, only outcome assessors and data analysts will be blinded.

Research methodology

The anthropometric parameters, including body weight, body height, waist and hip circumferences, will be measured, and BMI and waist-to-hip ratio (WHR) will be calculated pre- and post-intervention. Self-measured body weight will also be monitored monthly during the intervention. Body composition (the percentage of fat (%FM) and free-fat mass (%FFM) for the total body and individual body regions (arms, trunk, legs, head), male-specific (android) and female-specific (gynoid) area, visceral adipose tissue (VAT), appendicular lean mass index (ALMI) and lean mass index (LMI)) and densitometric parameters (BMD and BMC at the lumbar spine (L1-L4), femoral neck and total body) will be assessed before and after the intervention using dual-energy X-ray absorptiometry methods utilising the DEXA Hologic QDR Discovery (Bedford, Massachusetts, USA) analyser. Pre- and post-intervention systolic (SBP) and diastolic blood pressure (DBP) will be measured using an electronic blood pressure monitor (Omron M2, Kyoto, Japan). Prior to and following the intervention the following biochemical parameters will be assessed: osteocalcin, bone-specific alkaline phosphatase (BSAP), cross-linked C-terminal telopeptide of type I collagen (CTX-I), fasting glucose and insulin, TC, low-density LDL-C, high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), asymmetric dimethylarginine (ADMA), soluble vascular cell adhesion molecule-1 (sVCAM-1), high-sensitivity C-reactive protein (hs-CRP), 25-hydroxyvitamin D (25(OH)D) and AGEs levels. Moreover, follicle-stimulating, luteinising, oestradiol, progesterone and parathyroid hormones levels will be measured during the enrolment phase to confirm participants' menopausal status and to exclude underactive or overactive parathyroid glands. Glucose, insulin, lipid profile, hsCRP, osteocalcin, BSAP, 25(OH)D and hormones will be measured in the commercial laboratory (Diagnostyka sp. z o.o., Życzkowskiego 16 Str., Cracow, Poland) using standard laboratory procedures and other parameters will be evaluated in the Laboratory of the Department of Pediatric Gastroenterology and Metabolic Diseases using enzyme-linked immunosorbent assay method. Homeostasis model assessment of insulin resistance (HOMA-IR) and quantitative insulin sensitivity check index (QUICKI) will be calculated. The intake of different food groups will be evaluated using the Dietary Habits and Nutrition Beliefs Questionnaire [30] before, during and after the intervention. Dietary habits will also be assessed using 3-day dietary records before and during the intervention to check protocol requirements using the Aliant software (Anmarsoft, Gdańsk, Poland). AGEs contents in diet will be estimated using Uribarri et al. [5] database. Physical activity will be determined before, during and after the intervention using the International Physical Activity Questionnaire [31]. Study participants will also be asked to complete medical history and socio-economic questionnaires before and after the intervention. The study design is illustrated in Figure 1.

The study's primary outcomes will include changes (post- minus pre-intervention values) in BMD, BMC, osteocalcin, BSAP and CTX-I levels. The secondary outcomes will be changes in fasting glucose and insulin, HOMA-IR, QUICKI, TC, LDL-C, HDL-C, TG, ADMA, sVCAM-1, hs-CRP, SBP, DBP, body weight, waist, hip circumference, BMI, %FM, %FFM, VAT, ALMI, LMI, AGEs, 25(OH)D.

Python (pandas, scipy and numpy libraries) will be used for all data analysis. A two-sided p-value < 0.05 will be considered statistically significant. The overall characteristics of subjects will be expressed as means and standard deviations with a 95% confidence interval if the data is normally distributed; otherwise, data will be expressed as medians and interquartile ranges. The normality of the distribution of the variable will be verified using the Shapiro-Wilk test of normality. Comparisons between two unpaired groups will be determined using t-tests or Mann-Whitney U tests. The paired t-test or Wilcoxon test will be used to analyse the statistical significance of the pre- and post-intervention variables. Moreover, a generalised linear model will be used to analyse the influence of the intervention on analysed parameters. Contingency tables will be used to assess relationships between categorical variables. Depending on data distribution, parametric (Pearsons) or nonparametric tests (Spearman rank) will be applied to assess correlations. Uni-

and multivariate logistic and linear regression analyses will be performed.

Measurable effects and expected results

It has been shown that subjects with osteoporosis have higher serum AGEs levels than healthy individuals and serum AGEs concentrations are negatively correlated with BMD [16]. AGEs have also significantly inhibited osteoblast proliferation, differentiation and mineralisation and induced osteoblast apoptosis [14]. These observations suggest that AGEs may play an important role in bone health. Moreover, several studies reported that a low AGE diet might improve anthropometric parameters, body composition, and lipid and inflammatory profiles [21–25].

This is the first randomised controlled trial to assess the effect of low AGEs diet on BMD and BMC, selected markers of bone formation and resorption in postmenopausal overweight and obese women. The study also fills gaps in the knowledge of the effect of a low AGEs diet on cardio-metabolic parameters. The results of this study should give a better insight into the effect of a low AGEs diet on bone health and cardio-metabolic parameters in overweight and obese postmenopausal women.

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Conflict of interest statement
The authors declare no conflict of interest.

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References

1. World Health Organization. Menopause. [cited 2022 Dec 6]. Available from: https://www.who.int/news-room/fact-sheets/detail/menopausel.
2. Roa-Díaz ZM, Raguindin PF, Bano A, Laine JE, Muka T, Glisic M. Menopause and cardiometabolic diseases: What we (don't) know and why it matters. Maturitas. 2021 Oct;152:48-56. doi: 10.1016/j.maturitas.2021.06.013.
3. Kim KC, Shin DH, Lee SY, Im JA, Lee DC. Relation between obesity and bone mineral density and vertebral fractures in Korean postmenopausal women. Yonsei Med J. 2010 Nov;51(6):857-63. doi: 10.3349/ymj.2010.51.6.857.
4. Uribarri J, Cai W, Sandu O, Peppa M, Goldberg T, Vlassara H. Diet-derived advanced glycation end products are major contributors to the body's AGE pool and induce inflammation in healthy subjects. Ann N Y Acad Sci. 2005 Jun;1043:461-6. doi: 10.1196/annals.1333.052.
5. Uribarri J, Woodruff S, Goodman S, Cai W, Chen X, Pyzik R, Yong A, Striker GE, Vlassara H. Advanced glycation end products in foods and a practical guide to their reduction in the diet. J Am Diet Assoc. 2010 Jun;110(6):911-6. doi: 10.1016/j.jada.2010.03.018.
6. Thomas MC, Woodward M, Neal B, Li Q, Pickering R, Marre M, Williams B, Perkovic V, Cooper ME, Zoungas S, Chalmers J, Hillis GS; ADVANCE Collaborative Group. Relationship between levels of advanced glycation end products and their soluble receptor and adverse outcomes in adults with type 2 diabetes. Diabetes Care. 2015 Oct;38(10):1891-7. doi: 10.2337/dc15-0925.
7. Kerkeni M, Weiss IS, Jaisson S, Dandana A, Addad F, Gillery P, Hammami M. Increased serum concentrations of pentosidine are related to presence and severity of coronary artery disease. Thromb Res. 2014 Sep;134(3):633-8. doi: 10.1016/j.thromres.2014.07.008.
8. Angoorania P, Ejtahedbc HS, Mirmiranad P, Mirzaeia S, Azizi F. Dietary consumption of advanced glycation end products and risk of metabolic syndrome. Int J Food Sci Nutr. 2016;67:170-6. doi: 10.3109/09637486.2015.1137889.
9. Yamagishi S, Nakamura K, Inoue H, Kikuchi S, Takeuchi M. Serum or cerebrospinal fluid levels of glyceraldehyde-derived advanced glycation end products (AGEs) may be a promising biomarker for early detection of Alzheimer's disease. Med Hypotheses. 2005;64:1205-7. doi: 10.1016/j.mehy.2005.01.016.
10. Jiao L, Taylor PR, Weinstein SJ, Graubard BI, Virtamo J, Albanes D, Stolzenberg-Solomon RZ. Advanced glycation end products, soluble receptor for advanced glycation end products, and risk of colorectal cancer. Cancer Epidemiol Biomarkers Prev. 2011 Jul;20(7):1430-8. doi: 10.1158/1055-9965.EPI-11-0066.
11. Tantalaki E, Piperi C, Livadas S, Kollias A, Adamopoulos C, Koulouri A, Christakou C, Diamanti-Kandarakis E. Impact of dietary modification of advanced glycation end products (AGEs) on the hormonal and metabolic profile of women with polycystic ovary syndrome (PCOS). Hormones (Athens). 2014 Jan-Mar;13(1):65-73. doi: 10.1007/BF03401321.

12. Semba RD, Bandinelli S, Sun K, Guralnik JM, Ferrucci L. Plasma carboxymethyl-lysine, an advanced glycation end product, and all-cause and cardiovascular disease mortality in older community-dwelling adults. J Am Geriatr Soc. 2009 Oct;57(10):1874-80. doi: 10.1111/j.1532-5415.2009.02438.x.
13. Yamamoto M, Sugimoto T. Advanced glycation end products, diabetes, and bone strength. Curr Osteoporos Rep. 2016 Dec;14(6):320-6. doi: 10.1007/s11914-016-0332-1.
14. Ge W, Jie J, Yao J, Li W, Cheng Y, Lu W. Advanced glycation end products promote osteoporosis by inducing ferroptosis in osteoblasts. Mol Med Rep. 2022 Apr;25(4):140. doi: 10.3892/mmr.2022.12656.
15. Saito M, Marumo K. Collagen cross-links as a determinant of bone quality: a possible explanation for bone fragility in aging, osteoporosis, and diabetes mellitus. Osteoporos Int. 2010 Feb;21:195-214. doi: 10.1007/s00198-009-1066-z.
16. Yang DH, Chiang TI, Chang IC, Lin FH, Wei CC, Cheng YW. Increased levels of circulating advanced glycation end-products in menopausal women with osteoporosis. Int J Med Sci. 2014 Mar;11(5):453-60. doi: 10.7150/ijms.8172.
17. Kindler JM, Laing EM, Liu W, Dain JA, Lewis RD. Pentosidine is associated with cortical bone geometry and insulin resistance in otherwise healthy children. J Bone Miner Res. 2019 Aug;34(8):1446-50. doi: 10.1002/jbmr.3727.
18. Illien-Jünger S, Palacio-Mancheno P, Kindschuh WF, Chen X, Sroga GE, Vashishth D, Iatridis JC. Dietary advanced glycation end products have sex- and age-dependent effects on vertebral bone microstructure and mechanical function in mice. J Bone Miner Res. 2018 Mar;33(3):437-48. doi: 10.1002/jbmr.3321.
19. Carnovali M, Luzi L, Terruzzi I, Banfi G, Mariotti M. Metabolic and bone effects of high-fat diet in adult zebrafish. Endocrine. 2018 Aug;61(2):317-26. doi: 10.1007/s12020-017-1494-z.
20. Waqas K, Chen J, van der Eerden BCJ, Ikram MA, Uitterlinden AG, Voortman T, Zillikens MC. Dietary advanced glycation end-products (dAGEs) intake and bone health: a cross-sectional analysis in the Rotterdam study. Nutrients. 2020 Aug;12(8):2377. doi: 10.3390/nu12082377.
21. Sohouli MH, Fatahi S, Sharifi-Zahabi E, Santos HO, Tripathi N, Lari A, Pourrajab B, Kord-Varkaneh H, Găman MA, Shidfar F. The impact of low advanced glycation end products diet on metabolic risk factors: a systematic review and meta-analysis of randomized controlled trials. Adv Nutr. 2021 Jun;12(3):766-76. doi: 10.1093/advances/nmaa150.
22. Sohouli MH, Sharifi-Zahabi E, Lari A, Fatahi S, Shidfar F. The impact of low advanced glycation end products diet on obesity and related hormones: a systematic review and meta-analysis. Sci Rep. 2020 Dec;10(1):22194. doi: 10.1038/s41598-020-79216-y.
23. Baye E, de Courten MP, Walker K, Ranasinha S, Earnest A, Forbes JM, de Courten B. Effect of dietary advanced glycation end products on inflammation and cardiovascular risks in healthy overweight adults: a randomised crossover trial. Sci Rep. 2017;7(1):4123.
24. Poulsen MW, Bak MJ, Andersen JM, Monošík R, Giraudi-Futin AC, Holst JJ, Nielsen J, Lauritzen L, Larsen LH, Bügel S, Dragsted LO. Effect of dietary advanced glycation end products on postprandial appetite, inflammation, and endothelial activation in healthy overweight individuals. Eur J Nutr. 2014;53(2):661-72. doi: 10.1007/s00394-013-0574-y.
25. Semba RD, Gebauer SK, Baer DJ, Sun K, Turner R, Silber HA, Talegawkar S, Ferrucci L, Novotny JA. Dietary intake of advanced glycation end products did not affect endothelial function and inflammation in healthy adults in a randomized controlled trial. J Nutr. 2014 Jul;144(7):1037-42. doi: 10.3945/jn.113.189480.
26. Kahleova H, Znayenko-Miller T, Uribarri J, Schmidt N, Kolipaka S, Hata E, Holtz DN, Sutton M, Holubkov R, Barnard ND. Dietary advanced glycation end-products and postmenopausal hot flashes: a post-hoc analysis of a 12-week randomized clinical trial. Maturitas. 2023 Jun;172:32-8. doi: 10.1016/j.maturitas.2023.03.008.
27. Chan AW, Tetzlaff JM, Altman DG, Laupacis A, Gøtzsche PC, Krleža-Jerić K, Hróbjartsson A, Mann H, Dickersin K, Berlin JA, Doré CJ, Parulekar WR, Summerskill WS, Groves T, Schulz KF, Sox HC, Rockhold FW, Rennie D, Moher D. SPIRIT 2013 statement: defining standard protocol items for clinical trials. Ann Intern Med. 2013 Feb;158(3):200-7. doi: 10.7326/0003-4819-158-3-201302050-00583.
28. Chan AW, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin JA, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleza-Jeric K, Laupacis A, Moher D. SPIRIT 2013 explanation and elaboration: guidance for protocols of clinical trials. BMJ. 2013 Jan;346:e7586. doi: 10.1136/bmj.e7586.
29. Sawicka-Gutaj N, Gruszczyński D, Guzik P, Mostowska A, Walkowiak J. Publication ethics of human studies in the light of the Declaration of Helsinki – a mini-review. JMS. 2022 Jun;91(2):e700. doi: 10.20883/medical.e700.
30. Jezewska-Zychowicz M, Gawecki J, Wadolowska L, Czarnocinska J, Galinski G, Kollajtis-Dolowy A, et al. KomPAN® Dietary Habits and Nutrition Beliefs Questionnaire for adolescents aged 16-18 years and adults, version 2.1. – interviewer administered questionnaire. Chapter 1. (in:) KomPAN® Dietary Habits and Nutrition Beliefs Questionnaire and the manual for developing of nutritional data. Gawecki J, editor. Olsztyn: The Committee of Human Nutrition, Polish Academy of Sciences; 2024. 4-21 p.
31. Biernat E. International Physical Activity Questionnaire - Polish long version. Polish J Sport Med. 2013;29(1(4)):1-15.

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Acknowledgements

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1. Phillips SJ, Whisnant JP. Hypertension and stroke. In: Laragh JH, Brenner BM, editors. Hypertension: pathophysiology, diagnosis, and management. 2nd ed. New York: Raven Press; 1995. p. 465–478.

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