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SECRETARIAT ADDRESS
27/33 Szpitalna Street, 60-572 Poznań, Poland
phone: +48 618491432, fax: +48 618472685
e-mail: jms@ump.edu.pl
www.jms.ump.edu.pl

DISTRIBUTION AND SUBSCRIPTIONS
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phone/fax: +48 618547414
e-mail: sprzedazwydawnictw@ump.edu.pl

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WYDAWNICTWO NAUKOWE
UNIWERSYTETU MEDYCZNEGO
IM. KAROLA MARCINKOWSKIEGO
W POZNANIU

60-812 Poznań, ul. Bukowska 70
tel./fax: +48 618547151
www.wydawnictwo.ump.edu.pl

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The Editorial Board kindly informs that since 2014 *Nowiny Lekarskie* has been renamed to *Journal of Medical Science*.

The renaming was caused by using English as the language of publications and by a wide range of other organisational changes. They were necessary to follow dynamic transformations on the publishing market. The Editors also wanted to improve the factual and publishing standard of the journal. We wish to assure our readers that we will continue the good tradition of *Nowiny Lekarskie*.

You are welcome to publish your basic, medical and pharmaceutical science articles in *Journal of Medical Science*.

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ORIGINAL PAPER

JMS Journal of Medical Science

Comparison of Hand Eczema Search Terms in Iraq Before and During SARS-CoV-2 Pandemic Using Frequentist Statistics and Polynomial Models

Ahmed Al-Imam

Department of Computer Science and Statistics, Faculty of Medicine, PUMS Doctoral School, Poznan University of Medical Sciences, Poland

Department of Anatomy and Cellular Biology, College of Medicine, University of Baghdad, Iraq

Barts and The London School of Medicine and Dentistry, Queen Mary University of London, the United Kingdom.

https://orcid.org/0000-0003-1846-9424

Corresponding author: ahmed.al.imam@student.ump.edu.pl

Nada Al-Ward

Department of Community Medicine, College of Medicine, University of Baghdad, Iraq

Middle East Regional Office, World Health Organization

alwardn@yahoo.com

Manal M. Younus

Iraqi Pharmacovigilance Center, Ministry of Health, Iraq

Middle East Chapter, International Society of Pharmacovigilance

https://orcid.org/0000-0001-9475-5700

Omar Aimer

International Society of Pharmacovigilance, Canada

https://orcid.org/0000-0002-0874-486X

Ali K. Al-Shalchy

Neurosurgical Unit, Department of Surgery, College of Medicine, University of Baghdad, Iraq; The Royal College of Surgeons, United Kingdom

ali_alshalchy@yahoo.com

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ABSTRACT

Introduction. SARS-CoV-2 pandemic spread around the world exponentially. People use disinfectants excessively as a form of protection from the novel coronavirus, which may result in contact eczema. This, in turn, may be monitored by the local health authorities. Our study explores the internet in order to detect significant changes in online information search behaviors associated with eczema in Iraq during the pandemic.

Material and Methods. We searched the internet, via Google Trends, using five search terms; "اكزيما", "الاكزيما", "اكزيما اليد", "كحول", and "مطهر"; these are the Arabic translation for "eczema", "the eczema", "hand eczema", "alcohol", and "disinfectant". We explored the temporal mapping covering two years, before and during the pandemic, using frequentist statistics, polynomial models, and neural networks to evaluate the time series which reflects web users' information-seeking behavior with regard to these terms.

Results. Spatial mapping conveyed data from six Iraq governorates, including Ninawa, Babil, Al-Najaf, Baghdad, Basrah, and Erbil. Basrah governorate had the highest score (interest) for the search term "the eczema" (الاكزيما), while Al-Najaf had the highest score regarding the search term "disinfectant" (مطهر). Tempo-

ral mapping exhibited high variability, the highest of which was for the "the eczema" (الاكزيما) and "alcohol" (كحول). Exploring the time series using polynomial models demonstrated a weak power over the two years. However, in the course of the pandemic year, all models possessed moderate power.
Conclusions. Changes in the human behavior during pandemic events are of prime importance for the pharmacovigilance experts. Pandemics may affect medical conditions, including hand eczema, as a manifestation of disinfectants overuse. Combining statistics and artificial intelligence facilitates screening, detecting, and collecting pharmacovigilance safety data.

Introduction

Pharmacovigilance comprises the science and activities of detecting, assessing, understanding, and preventing adverse effects of drugs or drug-related problems [1]. Since its global expansion in the 1960s, following the thalidomide tragedy and for many drug safety interventions, such as drug withdrawals, labeling changes, and prescription restrictions, the aforementioned branch of science shows an exponential progression and importance in health programs. This, in turn, has led, over the last decade, to a fast evolution of regulation with more requirements from health authorities [2, 3]. In fact, the advancement of the internet, social media, and digital health data contributed to an additional increase of safety and effectiveness data, and have been accelerated by the current global pandemic due to the novel coronavirus 2019 (2019-nCoV). Additionally, the collection, detection, and assessment of the increasing safety data can be facilitated using artificial intelligence.

Since the initial reports of the Wuhan outbreak, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has been spreading around the world at an alarmingly exponential rate [4, 5]. As of 25 February 2022, the confirmed infections exceeded 432,505,813 worldwide and 2,299,767 in Iraq [4]. Complications related to the illness claimed the lives of over 5,950,376 globally and 24,948 in Iraq [4]. Furthermore, SARS-CoV-2 affected nations from developed and developing countries; the most affected to date in total (cumulative) cases included the United States, India, Brazil, France, United Kingdom, Russia, Germany, Turkey, Italy, and Spain [4]. As a top priority for the global health agenda, scientists successfully developed several effective vaccines, including Pfizer–BioNTech (BNT162b2), Moderna (mRNA-1273), and AstraZeneca (AZD1222) [6-8]. However, novel mutant strains of the coronavirus disease 2019 (COVID-19) emerged, including those discovered in the United Kingdom (α variant), the delta variant (δ) in India, and the most recent omicron (O) variant in South Africa [9]. Until the vaccination campaign is extended to the entire world population and novel variants of the virus stopped to emerge – which is unlikely – the first preventive measures, such as social distancing, personal protective equipment, and hand disinfection must be maintained. In fact, new variants may persistently emerge and evade vaccine-induced immunity and the proclaimed natural "herd immunity".

People worldwide have been using disinfectants, including alcohol, as a form of protection from the novel coronavirus. Eczema (dermatitis), specifically contact eczema of the hand, are dermatological conditions that can manifest due to the overzealous use of those chemicals; nonetheless, Minamoto et al. (2016) carefully cited that chemical additives – such as sodium laureth sulfate (SLES) – may account for inducing an eczematous skin reaction, rather than alcohol itself [10]. Eczema may manifest as an acute or chronic condition, which mandates medical treatment to prevent subsequent complications, including those affecting the manual skills, which in turn may drastically impair the quality of life, particularly in the elderly [11]. We refer to everyday activities where we use our hands to manipulate physical objects, write, draw, and many other functions. The hand represents the prehensile, or grasping "organ", of human beings and primates. Therefore, the conditions affecting the musculoskeletal or motor anatomical units of the hand, its skin, or the haptic sensory feedback mechanisms may handicap, or reduce the functional capacity of the hand and its derived manual skills.

Our study aims to explore the internet by searching for adverse events (AE) terms related

to eczema, dermatitis, and disinfectants, in order to investigate the specific spatial-temporal patterns before and during the pandemic. Our primary objective is to detect significant changes in web search queries connected with eczema in Iraq that may relate to the overzealous use of disinfectants during the pandemic. We believe that the current study is essential for health officials at the Iraqi Ministry of Health and local pharmacovigilance authority. Using frequentist inference and artificial intelligence methods, our study may provide novel insights on the importance of the digital knowledge and safety data available via the world wide web to predict future trends during the pandemic, parallel phenomena, as well as safety surveillance of the pharmaceutical products [12-15].

Material and Methods

We searched the internet, via Google Trends, using five search terms: "اكزيما", "الاكزيما", "اكزيما اليد", "كحول", and "مطهر" [16]. These terms are the Arabic language translation for "eczema", "the eczema", "hand eczema", "alcohol", and "disinfectant". We explored the web in the span of two years, one year before and one year during the pandemic, from 17th November 2018 to 17th November 2020, in order to compare the pre-pandemic versus the pandemic year (era) in connection with our study objectives. The date 17th November 2019 marks the emergence of SARS-CoV-2 in Wuhan in China [4, 5]. The authors know that the term "alcohol" is not limited to alcohol used for medicinal purposes and disinfection, and thus can also refer to alcoholic drinks and beverages. However, from the cultural standpoint, Iraqis do not refer to the latter category as "alcohol". Instead, they would rather refer to brand names, commercial, and public names from their native language.

We deployed the Shapiro-Wilk test to evaluate the normality distribution. Spearman's rank-order correlation was used to test potential significant correlations among search terms (web queries). The independent samples Mann-Whitney U test (nonparametric testing) compared the pre-pandemic versus the pandemic year. Polynomial modeling for each search term was conveyed to anticipate if the model properly fits the time series, representing web users' online information search behavior concerning the five terms of interest. A calculated probability of less than 0.05 (p-value < 0.05) was considered the cut-off margin for statistical significance. We processed the raw data with Microsoft Excel 2016 with Analysis ToolPak add-in, and we conducted data analytics and machine learning with IBM Statistical Package for the Social Sciences (SPSS) version 26.

We implemented the supervised machine learning using multilayer perceptron (MLP) neural networks based on the scaled conjugate gradient optimization algorithm and a default SPSS allocation of the training set and testing set at 70% and 30%, respectively. The MLP procedure uses a feedforward architecture – data only moves from input nodes through the hidden layer of nodes to output nodes – to produce a model for dependent (outcome) variables based on the values of one or more independent variables (predictors). The feedforward neural network represents a prototypical form of artificial neural networks (ANN). MLP generates synaptic weights and independent variables importance analysis. As opposed to the descendant and recurrent neural networks, in feedforward ANN connections between the nodes do not form a cycle.

Concerning the raw data, there were enough data points for each variable to train the neural network without overfitting. When considering the holistic period, there were 105 entries (data points) and 52 versus 53 data points for the pre-pandemic versus the pandemic period. Nonetheless, overfitting may also occur, as we consider the case of Iraq, not an aggregate of countries. The temporal resolution was one week at a time (weekly intervals); exploring Google Trends on a year-by-year basis provided the weekly-based temporal intervals.

The research did not mandate ethical approval, since we worked on open data publicly available on the internet via the Google Trends engine, and the study involves no patients.

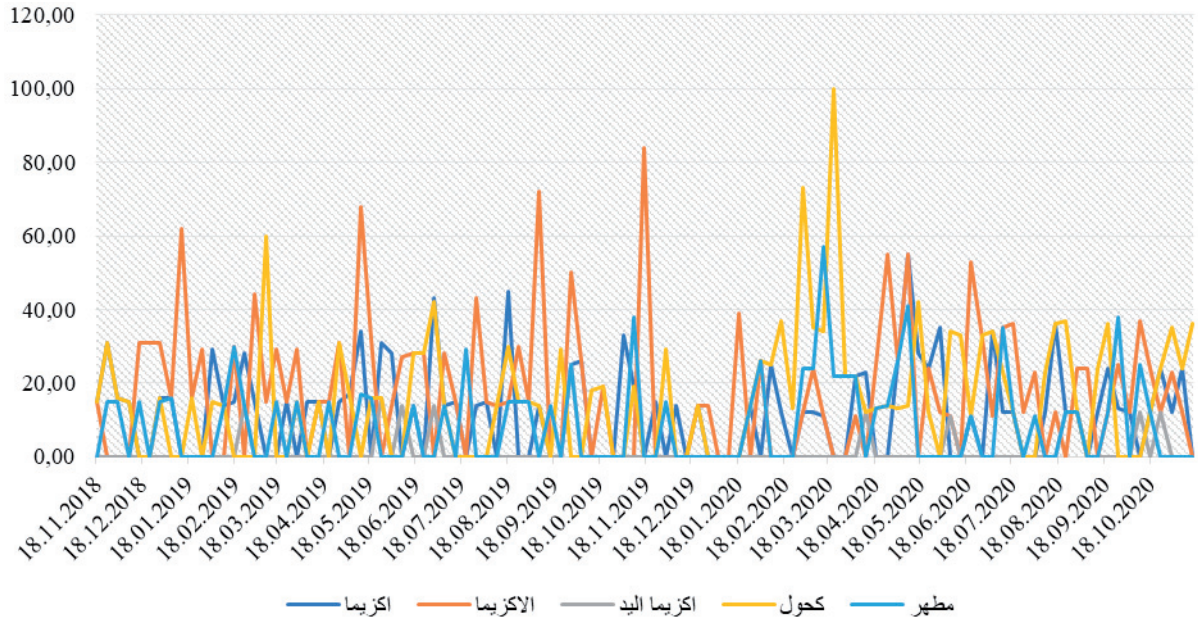
Results

Geographic (spatial) mapping, based on the analysis of the raw data retrieved from Google Trends website revealed forty countries with interest in the topic of the AE term "eczema" (اكزيما), the top

twenty of which included France, Malaysia, Singapore, Pakistan, Switzerland, Nigeria, Italy, Vietnam, Brazil, Saudi Arabia, New Zealand, United Kingdom, Kenya, Canada, Philippines, Hong Kong, Bangladesh, Australia, United Arab Emirates, and Ireland. Only three countries from the Arab world contributed to the spatial map, involving Saudi Arabia, United Arab Emirates, and Egypt. The top ten countries of highest interest in disinfectants comprised Brazil, Japan, Indonesia, Italy, Saudi Arabia, Egypt, Germany, Bangladesh, Switzerland, and Hong Kong. Iraq's spatial mapping conveyed data from six governorates, including Ninawa, Babil, Al-Najaf, Baghdad, Basrah, and Erbil. In contrast, data connected with the search term "اكزيما اليد" (hand eczema) were completely absent due to the inherent limitations of Google Trends itself. Basrah governorate had the highest score (interest) for the search term "الاكزيما" (the eczema), while Al-Najaf had the highest score for the search term "مطهر" (disinfectant).

On the other hand, temporal mapping (Figure 1) exhibited high variability, the highest of which was for the search terms "الاكزيما" (the eczema) (mean = 17.67 +/- standard error of mean = 1.773) and "كحول" (alcohol) (15.58 +/- 1.655), while the lowest of which was for the search terms "اكزيما" (eczema) (12.12 +/- 1.211), "مطهر" (disinfectant) (8.49 +/- 1.141), and "اكزيما اليد" (hand eczema) (1.47 +/- 0.437). We deployed polynomial modeling to explore the temporal variabilities within the time series for each search term (**Table 1**); we interpreted the strength of the correlation between time (x-axis, predictor variable), as well as the online information search behavior (y-axis, dependent variable) per the correlation coefficient. For the holistic period (two years), all models had a weak power with an exception for the model describing the temporal mapping of the search term "كحول" (alcohol) (R^2 score = 0.164; Correlation Coefficient = 0.406). Similarly, during the pre-pandemic year, all search terms presented models of weak power except for the search term "الاكزيما" (the eczema) (R^2 score = 0.109; Correlation Coefficient = 0.330). However, during the pandemic year, the models for each search term showed a moderate power (**Table 1**).

Using the Shapiro-Wilk test, none of the search terms had a normal distribution (p-value < 0.001), and the statistical outliers existed during February 2019, May and June 2019, August and September 2019, November 2019, February to June 2020, and August to October 2020. The highest number of statistical outliers was for the search term "اكزيما اليد" (hand eczema), and these were mainly clustered in the pandemic during April



* x = time, y = interest over the surface web.
** Pre-Pandemic (17th November 2018 to 17th November 2019), and pandemic (17th November 2019 to 17th November 2020).

Figure 1. Temporal Mapping of the Online Information Search Behaviour

Table 1. Polynomial Models

Era	Parameter	Order of Polynomial Function	R^2 Score	Correlation Coefficient	Correlation Coefficient Strength
Pre-Pandemic & Pandemic	كحول (alcohol)	6^{th}	0.164	0.406	Moderate
	اكزيما (eczema)	6^{th}	0.058	0.241	Weak
	اكزيما اليد (hand eczema)	6^{th}	0.049	0.221	Weak
	(disinfectant)	6^{th}	0.040	0.199	Weak
	الاكزيما (the eczema)	6^{th}	0.039	0.198	Weak
Pre-Pandemic	الاكزيما (the eczema)	6^{th}	0.109	0.330	Moderate
	اكزيما (eczema)	6^{th}	0.072	0.296	Weak
	مطهر (disinfectant)	6^{th}	0.080	0.283	Weak
	كحول (alcohol)	6^{th}	0.056	0.236	Weak
	اكزيما اليد (hand eczema)	6^{th}	0.052	0.229	Weak
Pandemic	كحول (alcohol)	6^{th}	0.258	0.508	Moderate
	مطهر (disinfectant)	6^{th}	0.234	0.484	Moderate
	الاكزيما (the eczema)	6^{th}	0.176	0.420	Moderate
	اكزيما (eczema)	6^{th}	0.1405	0.375	Moderate
	اكزيما اليد (hand eczema)	6^{th}	0.123	0.351	Moderate

* x = time, y = interest over the surface web.
** Pre-Pandemic (17^{th} November 2018 to 17^{th} November 2019), and pandemic (17^{th} November 2019 to 17^{th} November 2020).

2020, June 2020, August 2020, and October 2020. For the holistic period, bivariate correlations confirmed the existence of a statistically significant correlation for "الاكزيما" (the eczema) versus "مطهر" (disinfectant) (Spearman's rho Correlation Coeffecient = 0.221, p-value = 0.024) and "اكزيما" (the eczema) versus "كحول" (alcohol) (-0.268, 0.006). Nonetheless, each of these two correlations had a weak effect size. During the pre-pandemic period, there was only one significant correlation, although also of weak effect size, for the search term "اكزيما" (eczema) versus "اكزيما اليد" (hand eczema) (Spearman's rho Correlation Coeffecient = 0.291, p-value = 0.037). For the pandemic period, there was a statistically significant correlation for "الاكزيما" (the eczema) versus "مطهر" (disinfectant) (Spearman's rho Correlation Coeffecient = 0.359, p-value = 0.008) and "الاكزيما" (the eczema) versus "كحول" (alcohol) (-0.271, 0.049), of moderate and weak effect size, respectively (**Table 2**). Furthermore, nonparametric testing via the Mann-Whitney U test for the pre-pandemic versus the pandemic year detected a significant difference for one search term only, "كحول" (alcohol), which is in favor of the pandemic year (p-value = 0.036) (**Table 3** and **Figure 2**).

Considering the previous statistical inference and the existing evidence within the dermatology literature [17, 18], it is plausible that a causal relationship exists; the pandemic event might have affected web users with regard to searching for those five specific search terms. We are assuming that web queries using the two search terms "كحول" (alcohol) and "مطهر" (disinfectant), represent or reflect the independent variables (predictors). In comparison, the remain-

ing three terms that are related to AE terms concerning eczema and hand eczema represent the dependent variables (outcomes). Accordingly, we conducted a supervised machine learning model (**Table 4**), via neural networks for the holistic period, for which, the predictors' importance analysis conveyed higher importance for "كحول" (alcohol) (importance = 0.569, normalized

Table 2. Nonparametric Bivariate Correlations: Pandemic Era

		اكزيما (eczema)	الاكزيما (the eczema)	اكزيما اليد (hand eczema)	كحول (alcohol)	مطهر (disinfectant)
اكزيما (eczema)	Correlation Coefficient	1.000	-.046	.030	.198	-.016
	Sig. (2-tailed)	.	.744	.831	.155	.907
	N	53	53	53	53	53
الاكزيما (the eczema)	Correlation Coefficient	-.046	1.000	-.026	-.271*	.359**
	Sig. (2-tailed)	.744	.	.853	.049	.008
	N	53	53	53	53	53
اكزيما اليد (hand eczema)	Correlation Coefficient	.030	-.026	1.000	.031	.012
	Sig. (2-tailed)	.831	.853	.	.823	.931
	N	53	53	53	53	53
كحول (alcohol)	Correlation Coefficient	.198	-.271*	.031	1.000	.162
	Sig. (2-tailed)	.155	.049	.823	.	.245
	N	53	53	53	53	53
مطهر (disinfectant)	Correlation Coefficient	-.016	.359**	.012	.162	1.000
	Sig. (2-tailed)	.907	.008	.931	.245	.
	N	53	53	53	53	53

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

Table 3. Hypothesis Test Summary: Pre-Pandemic vs. Pandemic

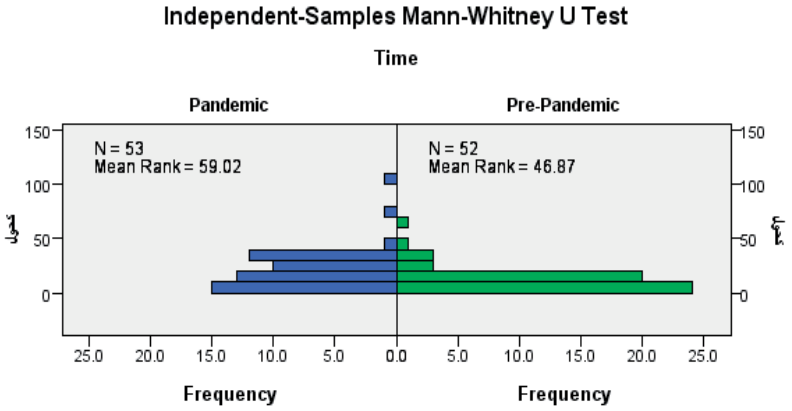
Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of اكزيما is the same across categories of Time.	Independent-Samples Mann-Whitney U Test	.272	Retain the null hypothesis.
2	The distribution of الاكزيما is the same across categories of Time.	Independent-Samples Mann-Whitney U Test	.177	Retain the null hypothesis.
3	The distribution of اكزيما اليد is the same across categories of Time.	Independent-Samples Mann-Whitney U Test	.918	Retain the null hypothesis.
4	The distribution of كحول is the same across categories of Time.	Independent-Samples Mann-Whitney U Test	.036	Reject the null hypothesis.
5	The distribution of مطهر is the same across categories of Time.	Independent-Samples Mann-Whitney U Test	.943	Retain the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

* Pre-Pandemic: 17th November 2018 to 17th November 2019 and Pandemic: 17th November 2019 to 17th November 2020
** اكزيما (eczema), الاكزيما (the eczema), اكزيما اليد (hand eczema), كحول (alcohol), مطهر (disinfectant)

importance = 100.0%) than "مطهر" (disinfectant) (0.431, 75.7%). In terms of the pre-pandemic year, the neural networks validated somewhat similar results for "كحول" (alcohol) (importance = 0.523, normalized importance = 100.0%) and "مطهر" (disinfectant) (0.477, 91%). As for the pandemic year, the independent variables' importance also assigned a weight that is higher for "كحول"



Total N	105
Mann-Whitney U	1,059.000
Wilcoxon W	2,437.000
Test Statistic	1,059.000
Standard Error	151.894
Standardized Test Statistic	-2.100
Asymptotic Sig. (2-sided test)	.036

* Pre-Pandemic: 17th November 2018 to 17th November 2019
** Pandemic: 17th November 2019 to 17th November 2020

Figure 2. Hypothesis Testing: Pre-Pandemic vs. Pandemic for Search Term "كحول" (alcohol)

Table 4. Supervised Machine Learning: Model Summary

Training	Sum of Squares Error		106.648
	Average Overall Relative Error		1.001
	Relative Error for Scale Dependents	اكزيما (eczema)	.994
		الاكزيما (the eczema)	.993
		اكزيما اليد (hand eczema)	1.017
	Stopping Rule Used		One consecutive step(s) with no decrease in error [a]
	Training Time		0:00:00.03
Testing	Sum of Squares Error		47.896
	Average Overall Relative Error		.981
	Relative Error for Scale Dependents	اكزيما (eczema)	.969
		الاكزيما (the eczema)	1.004
		اكزيما اليد (hand eczema)	.947

a – Error computations are based on the testing sample

(alcohol) (importance = 0.547, normalized importance = 100.0%) compared to "مطهر" (disinfectant) (0.453, 82.9%).

Discussion

The temporal mapping provided much data concerning our study objectives. Furthermore, we could map the temporal variations connected with the search terms with a higher temporal resolution, using polynomial models, during the pandemic. Bivariate correlations existed before and during the pandemic; however, the correlations were radically different. Contrary to our pre-study anticipation, AE search terms related to eczema and hand eczema did not significantly differ before and during the pandemic. Nevertheless, the web users searched significantly more for the term "كحول" (alcohol) during the pandemic. Thus, these results may indicate a significant increase in alcohol use for disinfection, although not a significant increase in eczema and hand eczema cases in Iraq during the pandemic. ANN analytics verified potential causality between the terms related to disinfectants as well as those related to the AE terms of eczema.

Statistical analyses for the pandemic period confirmed that there was a statistically significant correlation for "الاكزيما" (the eczema) versus "مطهر" (disinfectant) (Spearman's rho Correlation Coeffecient = 0.359, p-value = 0.008). Additionally, a significant increase was observed in the search volume for alcohol during the pandemic compared to the pre-pandemic. Furthermore, each of the polynomial models became stronger (moderate strength) when compared to the pre-pandemic, in which most of the models (4/5, 80%) had a weak strength (**Table 1**). Accordingly, the COVID-19 pandemic might have induced a change in human behavior, including online information search behavior reflected as the web search volume on the Google Trends website. Our study does not provide much concerning positive findings; besides, the calculated effect size was weak most of the time. Nonetheless, the effect size can vary from one country to another, bearing in mind the population size and other demographic parameters. Our research represents a piece of supporting evidence which may complement and guide future studies worldwide.

Machine learning is a subfield of computer science which learns patterns from data without providing explicit programming instructions to create algorithms intended to perform a specific task [12]. As a modality of narrow artificial intelligence (nAI), machine learning represents a powerful tool for researchers which can substantially reduce the considerable burden on the healthcare system and the economy during the pandemic [19]. Non-clinical research methods, such as machine learning, data mining, deep learning, and other nAI modalities may also facilitate the diagnosis and prognosis for SARS-CoV-2 pandemic patients. Few other studies discussed the condition of hand eczema during the COVID-19 pandemic, including Singh et al. (2020) and Bilcharz et al. (2020). In fact,they demonstarted that overzealous use of sanitizers and frequent hand washing might cause hand eczema due to a disrupted skin barrier. Additionally,they also reported an increasein the incidence of new-onset hand eczema within the general population due to the overzealous hand hygiene [17, 18]. In parallel with the previously mentioned studies, we emphasize the integration of ML and nAI with classical (frequentist) statistics for studying the digital epidemiology and the spatio-temporal analysis of the pandemic and collateral phenomena [20–24].

The rationale for implementing machine learning relies on five elements: (1) Collateral – supporting – evidence based on machine learning algorithms. (2) An alternative method to classical data analytics. (3) Reconciliation of frequentist inference with neural networks models. (4) A form of convergent thinking, dealing with digital epidemiological research questions from alternative perspectives. (5) A supporting method for future research in pharmacovigilance, pharmacotherapy, dermatological sciences, and digital epidemiology.

One of the study limitations is that the pandemic could have limited access to the healthcare institutions to some extent, and this would consequently result in the need to search for alternative solutions to health problems and diseases via the internet. Nonetheless, in Iraq, the healthcare system – as in all developing countries – is different and much inferior to that of the developed world, including Poland and the European Union. Another limitation was the inability to differentiate whether the search for alcohol was related

to medicinal alcohol, or to alcoholic beverages; hence, some false-positive results are possible. However, we believe that alcoholic drinkers in Iraq might search for alternate terms, such as "بيرة" (beer) and "عرك" (gin), rather than the term "كحول" (alcohol) per se. Moreover, another limitation is the absence of Arabic combined terminology for the search term "اكزيما اليد" (hand eczema), which also reflects the built-in limitations of the Google Trends website itself. We are aware that our study may only be considered as the supporting (collateral) evidence due to the nature of the study design, intrinsic (inherent) limitations of the Google Trends website, and the potentially non-specific mapping terms used to explore the spatiotemporal mapping of data from Google Trends. Therefore, subsequent robust pre-clinical and clinical research is mandatory to confirm our hypotheses and observations. Additionally, Google Trends also possesses inherent limitations and restrictions; for instance, the search results available through Google Trends are anonymous and only reflect individuals with internet access, potentially excluding specific groups of interest, for example, the elderly, terminally ill patients, the disabled, and underprivileged groups who lack access to the internet in low- and middle-income countries [25]. Furthermore, Google Trends only conveys relative numbers – a relative interest at a percentile scale – and there is no official way yet to access real absolute numbers. Google Trends also derive data from web users utilizing Google search engine only; therefore, web queries based on other search engines, including DuckDuckGo, Ecosia, Dogpile, WolframAlpha, Gigablast, Startpage, and Qwant, will not be mapped.

Our research has other limitations inherent to the observational study design, including a lower level of evidence than the experimental or quasi-experimental studies, hence it is prone to biases and confounding variables, and the reduced capacity to infer causality. Additionally, web users may avoid being tracked while surfing the web. Internet users can rely on internet protocol (IP) masking, virtual private networks (VPN), VPN applications, or utilizing web browsers and engines dedicated to anonymous internet use, including Tor browser and DuckDuckGo search engine [26]. Furthermore, web users in certain countries, such as the People's Republic of China (PRC), are obliged to use alternative national search engines due to the governmental policies and regulations; for instance, Baidu is the dominant internet search engine company in the PRC [27].

The findings can be summarized into 1) COVID-19 pandemic has impacted the manner in which disinfectants are used, which might result in adverse events, such as eczema and dermatitis due to disinfectants overuse, or because of chemical additives in alcohol. 2) Due to the pandemic, online information-seeking behavior has become more evident and manifested as Google Trends' search volume, which is one way to understand the magnitude of the problem. 3) Using artificial intelligence may facilitate the screening, detecting, and collecting safety data, extending to other health products.

During the pandemic, changes in human behavior, including online information search behavior, are of prime importance, not only to sociologists and psychologists, but also to physicians, epidemiologists, and pharmacovigilance specialists. Therefore, four key messages can be highlighted: 1) Pandemics, as rare events, might affect medical conditions which indirectly relate to the event itself, as in the case of hand eczema as a manifestation of disinfectants overuse. 2) Dermatologists can collaborate with data scientists to realize the applicability of big data for evidence-based pharmacovigilance. 3) Digital data are worthy of exploration from epidemiological and pharmacovigilance perspectives to guide the subsequent research of the higher level-of-evidence hierarchy, including randomized controlled trials and meta-analytic studies. 4) Our research methodology and results can be regarded as supporting evidence for the subsequent aggregate studies worldwide.

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Conflict of interest statement
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Availability of Data
Our data are available upon request from the corresponding author and within three years from the article's publication date.

Contribution of Authors
Ahmed Al-Imam (AA) collected the raw data, conceptualized the research and statistical hypotheses, conducted data analytics, and wrote the article's first draft. Nada Al-Ward (NAW), Manal M Younus (MMY), Omar Aimer (OA), and Ali K Al-Shalchy (AKA) contributed to revising the first draft, developing the discussion section, and enhancing its scholarly quality.

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ORIGINAL PAPER

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Effects of tizanidine premedication on the duration of perioperative maintenance dose of vecuronium bromide

Ceyhan Oflezer

Department of Anesthesiology, University of Health Sciences, Bakirkoy Training and Research Hospital for Psychiatry, Neurology and Neurosurgery, Istanbul/Turkey

https://orcid.org/0000-0001-5396-5939

Corresponding author: coflezer@yahoo.com

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ABSTRACT

Aim. The aim of this study was to evaluate the effects of preoperative administration of tizanidine on the maintenance dose duration of the nondepolarizing muscle relaxant, i.e. vecuronium bromide.

Material and Methods. This prospective clinical study was conducted in 30 adult male patients scheduled for elective inguinal hernia surgery. Patients were categorised into two randomised groups based on the premedication use. Group 1 included patients (n = 15) who received oral diazepam (Diazem) in the evening prior to the surgery, as well as meperidine (*Dolantin*) 1 mg/kg^{-1} (max. 50 mg) i.m. with 50 ml water by mouth 1 hour before the procedure. Group 2 comprised patients (n = 15) who were given oral tizanidine 4 mg in the evening before the surgery, as well as oral tizanidine 4 mg with 50 ml water 1 hour prior to the operation. The following parameters were recorded in both groups: pre-operative and perioperative diastolic arterial blood pressure, systolic arterial blood pressure, mean arterial blood pressure, heart rate, respiratory rate, pre-operative vecuronium bromide maintenance doses, procedure time and postoperative score according to the Ramsay scale in 1st, 2nd and 3rd hour.

Results. No significant difference was observed between the two groups with regard to the pre-operative and perioperative haemodynamic variables and the respiratory rate ($P > 0.05$), although it was found that vecuronium maintenance dose duration was significantly higher in the tizanidine premedicated group ($P = 0.015$). In addition, the operative time ($P = 0.128$) and the postoperative patients' Ramsay scores did not differ statistically between the two groups ($P > 0.05$).

Conclusions. The prolonged duration of vecuronium maintenance dose, the increased nondepolarizing block time, as well as haemodynamic stability preservation in patients undergoing inguinal hernia surgery following preoperative oral administration of tizanidine support the view that tizanidine can be used as an effective and safe myotonolytic premedication agent.

Introduction

Tizanidine, an imidazoline, is also an α_2 receptor agonist mainly derived from centrally acting myotonolytic clonidine. It is characterized by short duration of action, little effect on heart rate and blood pressure, as well as antinociceptive and

anticonvulsant effects [1]. Oral administration of tizanidine is quickly absorbed. It reaches its maximum plasma concentration in 0.75 to 2 hours. Since its elimination half-life is 2.1 to 4.2 hours, it may be applicable even in the day care surgery [2, 3]. The use of α_2-adrenergic agonists for the treatment of muscle spasticity is well known [4–6]. Tizanidine is a short-acting and potent agent which effectively reduces spasticity caused by multiple sclerosis, acquired brain injury or spinal cord injury [7, 8]. The most frequently reported adverse effects include dryness of the mouth, drowsiness, dizziness and fatigue, although it is generally a well tolerated agent. It has been shown that tizanidine has a similar clinical efficacy as baclofen and diazepam [9, 10]. Additionally, fewer treatment interruptions due to adverse events were also observed [11]. Tizanidine, which shows muscle relaxant, sedative and anxiolytic properties, is also used in the treatment of musculoskeletal pain [6]. Howerver, the role of tizanidine in the treatment of postoperative pain was investigated [12]. The use of tizanidine was supported in pain management studies, which inluded myofascial pain, neuropathic pain, chronic daily headache and low back pain [13–17]. Furthermore, tizanidine demonstrated fewer cardiovascular side effects, thus, making it an appealing drug to be used in anaesthesia [18]. There are several studies assessing the role of tizanidine as an effective anaesthetic premedication. In fact, oral premedication with tizanidine alleviates the hypertensive response during laryngoscopy, as well as reduces the induction dose of midazolam, the maintenance dose of propofol, and the minimum alveolar concentration of sevoflurane. Additionally, it prolongs spinal anaesthesia and reduces isoflurane consumption [1, 19–22]. Although tizanidine, which proved effective in spastic patients, was reported as a premedication in various studies, the findings with regard to its use with nondepolarizing drugs are scarce. Therefore, this study aimed to assess the effect of tizanidine premedication on the maintenance dose of vecuronium bromide, which is a neuromuscular blocking agent.

Material and Methods

30 male patients who were eligible for elective inguinal hernia surgery under general anaesthesia were included in this study. Patients were aged between 18–60 years and were assessed according to the American Society of Anesthesiologists Classification (ASA) 1, 2 and 3. Individuals with uncontrolled diabetes or hypertension, a history of cardiorespiratory, renal, endocrine, hepatic, neurological and psychiatric disorders, and those exposed to α_2 adrenergic agonists in the preceding two weeks were excluded from the study. Prior to the surgery, the patients were provided with a complete description of the study, and subsequently, an informed consent was obtained from each participant. Taksim Research and Training Hospital Local Ethical Committee approved the presented study.

Following the pre-anaesthetic examination, an 8-hour fast was recommended before the surgery. All patients were evaluated the night before the planned operation, and a full physical examination was performed. After brief information was given to the patients about the surgery, they were informed about the drugs used before the surgery, the anaesthesia method applied in the operating room and the TOF-Guard device (Biometer, Denmark). Patients were then divided into two randomised groups on the basis of the premedication use. Group 1 comprised patients (n = 15) who had received oral diazepam (Diazem) the evening before the operation and meperidine (Dolantin) 1 mg/kg^{-1} (max 50 mg) i.m. + 50 ml water per os 1 hour pre-operatively; Group 2 included patients (n = 15) who had oral tizanidine 4 mg administered in the evening before the operation and oral Tizanidine 4 mg + 50 ml water 1 hour pre-operatively. In addition, atropine 0.5 mg i.m. was administered to both groups as a standard measure. Preoperatively, arterial systolic blood pressure (SABP), arterial diastolic blood pressure (DABP), and mean arterial blood pressure (MABP), respiratory rates (RR), and heart rate (HR) values were recorded 3 minutes before the induction of general anaesthesia. The electrodes of the TOF-Guard device were placed on the ulnar nerve and the mechanical sensor was placed on the medial side of the patient's thumb and secured. Neuromuscular monitoring was undertaken using a Kontron Minimon (Kontron Instruments, Model Minimon, 7137 Plus, Charter Kontron, England) in the operating room. Following the intravenous administration of 1 mcg/kg^{-1} fentanyl (maximum 75 mcg/kg^{-1}), thiopental (Pentothal) 7 mg/kg^{-1} (maximum 500

mg) was administered and the TOF-Guard device was calibrated to 100%. Subsequently, muscle relaxation was induced with vecuronium bromide (Norcuron) 0.1 mg/kg^{-1}, the patient was intubated and delivered to the surgical team. All patients were operated by the same surgical team blinded to the medications involved in the study. Vital signs and sedation were assessed and recorded according to the Ramsay Scale (RSS). Maintenance of anaesthesia was facilitated using an inhaled mixture 65% N_2O-35% O_2 and isoflurane (Forane) 0.5–1%. During the operation, no other analgesic was administered. The depth of anaesthesia was provided with volatile anaesthetic dose changes according to the following vital signs, SABP, DABP, MABP, RR and HR, which had been recorded every 5 minutes pre-operatively. When the 3rd response of TOF was observed in the TOF-Guard device, the patients' muscle relaxant requirements were maintained using doses of 30 mcg/kg^{-1} in each patient. After the surgical team completed the procedure, the volatile, anaesthetic and N_2O gases were turned off and the patient's ventilation continued with the breathing circuit O_2. Each patient received 0.5 mg of atropine i.v. When the 3rd response was observed in the TOF-Guard device, neostigmine methyl sulfate (neostigmine) was administered i.v. for decurarization (1.5 mg). When the TOF response was 65% according to the TOF-Guard device, the patient was extubated. The patients were transferred to the recovery room and monitored for one hour. The sedation status of the patients was evaluated with a 6-stage Ramsay scale. According to the Ramsay scale, the scoring levels are the following: (1) Irritable, agitated and/or restless patient; (2) cooperative, oriented and calm; (3) only obeying orders; (4) sleeping, although responding immediately to glabella tapping or loud noise; (5) sleeping, with a slow response to tapping the glabella or loud noise; (6) no response at all to these stimuli. Our patients were evaluated with the Ramsay sedation score in the 1st, 2nd and 3rd postoperative hours.

Statistical Analysis

Statistical analyses were performed with the SPSS Version 18.0 (SPSS Inc., Chicago, IL, USA). The Mann-Whitney U test for continuous variables was used to evaluate differences between groups. Fisher's Exact and χ^2 tests were applied to analyse differences in proportions. A *P*-value of $P \leq 0.05$, was defined as statistically significant.

Results

When the ASA results of the patients were evaluated, 11 patients in Group 1 presented ASA score

Table 1. Comparison of clinical variables between the two groups

Variable (Mean ± SD)	Group 1 (*n* = 15)	Group 2 (*n* = 15)	Combined 1 & 2 (*n* = 30)	*P* Value
Age	49.87 ± 13.15	46.07 ± 15.94	47.97 ± 14.49	0.482
Weight	74.13 ± 7.5	71.07 ± 6.83	72.6 ± 7.22	0.252
Pre-op SABP	133 ± 18.11	134.33 ± 21.12	133.67 ± 19.34	0.854
Pre-op DABP	81.67 ± 11.13	84.47 ± 13.15	83.07 ± 12.05	0.534
Pre-op MABP	111 ± 14.93	107.80 ± 17.05	109.43 ± 15.83	0.581
Pre-op HR	79.47 ± 12.79	73.93 ± 5.71	75.93 ± 11.27	0.137
Pre-op RR	16.73 ± 2.74	16.87 ± 1.46	16.80 ± 2.16	0.869
Periop SABP	147.40 ± 16.19	141.80 ± 25.35	144.60 ± 27.09	0.477
Periop DABP	86.40 ± 11.43	82.13 ± 10.96	84.27 ± 11.21	0.306
Periop MABP	113.93 ± 14.82	107.60 ± 14.19	110.77 ± 14.61	0.240
Periop HR	79.60 ± 9.95	72.27 ± 11.64	76.70 ± 10.13	0.074
Periop RR	17.60 ± 3.64	16.80 ± 2.68	17.20 ± 3.17	0.499
Vec Int	35.53 ± 8.75	42.93 ± 6.75	39.23 ± 8.56	0.015*
Op Time	73.80 ± 20.28	85.53 ± 20.68	79.67 ± 20.99	0.128

Pre-op SABP: Pre-operative Systolic Arterial Blood Pressure; Pre-op DABP: Pre-operative Diastolic Arterial Blood Pressure; Pre-op MABP: Pre-operative Mean Arterial Blood Pressure; Pre-op HR: Pre-operative Peak Heart Rate; Pre-op RR: Pre-operative Respiratory Rates; Periop SABP: Peri-operative Systolic Arterial Blood Pressure; Periop DABP: Peri-operative Diastolic Arterial Blood Pressure; Periop MABP: Peri-operative Mean Arterial Blood Pressure; Periop HR: Peri-operative Peak Heart Rate; Periop RR: Peri-operative Respiratory Rates; Vec Int: Vecuronium maintenance dosage Interval; Op Time: Operation Time; P-values were calculated using Pearson χ^2 test; Level of significance set at $P \leq 0.05$

Table 2. Comparison of postoperative Ramsay sedation scores between the two groups

Time	Ramsey Score	Group 1		Group 2		P Value
		n	%	n	%	
First Hour	1	-	-	1	6.7	0.560
	2	3	20	5	33.3	
	3	10	66.7	8	53.3	
	4	2	13.3	1	6.7	
Second Hour	2	6	40	11	73.3	0.451
	3	9	60	4	26.7	
Third Hour	2	13	86.3	15	100	0.143
	3	2	13.7	-	-	

N: number of patients; %: percentage of group; P-values were calculated using Pearson χ^2 test; Level of significance set at $P \leq 0.05$

of I, three presented ASA II and one presented ASA III score; in Group 2, 9 patients showed ASA I score, and six - ASA II score. No significant differences were observed between the two groups (P = 0.332). There was also no statistically significant difference between the groups in terms of age (P = 0.482) and weight (P = 0.252). **Table 1** shows the statistical comparisons of haemodynamic variables, heart rates and respiratory rates between the two groups, pre-operatively and perioperatively. It was determined that the duration of the vecuronium maintenance dose was significantly prolonged in the tizanidine premedicated group (P = 0.015). However, the procedure time did not statistically differ between the two groups (P = 0.128). The comparison of the Ramsay sedation scores of the patients recorded at the first hour, second hour and third hour postoperatively are presented in **Table 2**. There were no significant differences in Ramsay sedation scores between the two groups, (P > 0.05).

Discussion

Methyldopa, an analogue of levodopa, enters the norepinephrine synthesis pathway and is converted to α-methylnorepinephrine and α-methylepinephrine. These pseudotransmitters activate α-adrenoceptors, in particular central α_2 receptors. As a result, norepinephrine is released and sympathetic tone decreases. Sympathetic tone is also reduced in patients receiving tizanidine, an α_2 agonist, due to the fat that the transmitters at the junctions in the spinal cord are adrenergic. Furthermore, studies in animal models also showed that tizanidine inhibits both mono- and polysynaptic reflexes [6, 23]. However, unlike baclofen, it reduces both mono- and polysynaptic excitation, although primarily monosynaptic excitations [6, 24]. Some researchers suggest that tizanidine inhibits polysynaptic reflexes [6, 25]. The result of this study might be accounted for by a decrease in the tone-providing impulse to the adductor pollicis. Tizanidine, which has a central alpha-2 noradrenergic agonist effect, decreases norepinephrine release and causes a decrease in the sympathetic tone, which initially might suggest that it potentiates muscle relaxation. However, the working principle of the TOF-Guard device in the study is supramaximal quadruple stimulus given at 10–12 second intervals, leading to severe muscle twitch of the adductor pollicis. Therefore, the decrease in tone in the muscle proximal to the electrodes could not be assessed due to the working principle of the device. The working mechanism of TOF-Guard device is designed according to the "all or nothing" principle of muscle contraction. If the TOF-Guard device could provide submaximal stimulation instead of supramaximal stimulation, the decrease in muscle tone would be significant. Moreover, submaximal stimulation could require impulses from the spinal cord providing the tone for muscle contraction.

Tizanidine, an imidazolidine derivative, is an effective alternative to oral clonidine [18]. In fact, the imidazolidine compounds related to clonidine and tizanidine are thought to inhibit acetylcholine transmitter release [26]. A study in this area using rabbit distal colon evaluated the potential effects on isotonic contraction. Extrinsic pelvic parasympathetic nerves were stimulated for 30 seconds at a frequency of 2 Hz. Atropine, when added to the medium, abolished the contractions induced by acetylcholine, but only partially

reduced the responses to the nerve stimulation. Clonidine and the related compounds (UK 14819, UK 14304, UK 15121, UK 11957 and UK 42620) inhibited nerve stimulation-induced contractions at concentrations which had no effect on exogenous acetylcholine responses. In our study, the imidazolide Tizadine [18], which also involves these metabolites, may have played a role in the prolongation of neuromuscular blockade. Since acetylcholine acts as a transmitter in the intestine and at the neuromuscular junction, tizanidine may also have direct effects on the neuromuscular junction [26]. However, to date no evidence is available to substantiate this thesis. During general anaesthesia, clonidine increases intraoperative circulatory stability by lowering catecholamine levels. This includes peripheral nerve blockade during regional anaesthesia. Clonidine prolongs the duration of the block, as it can alter the direct effects on the spinal cord through the postsynaptic α_2 receptors located in the dorsal horn. A reduction of post-operative tremor, inhibition of opioid-induced muscle stiffness, reduction of opioid withdrawal symptoms, and treatment of some chronic pain symptoms are among the areas where it is applicable. Its side effects comprise bradycardia, hypotension, sedation, respiratory depression and dryness of the mouth. Although methyldopa and clonidine are adrenergic agonists, they are also considered sympatholytic, since they reduce sympathetic release [27]. In this study, the effect of the first dose of vecuronium bromide was significantly prolonged in the tizanidine group. There is no known direct drug interaction between vecuronium bromide and tizanidine [27]. Thus, the absence of drug interaction may also indicate that the effect of tizanidine on prolonging neuromuscular block does not occur by binding to post junctional receptors at the neuro muscular junction where vecuronium bromide acts. Nonetheless, tizanidine does seem to prolong the effect of the neuromuscular block when the effects of neuromuscular blocking agents are monitored objectively using the TOF-Guard method. Therefore, there might be a direct action on the ulnar nerve, a peripheral nerve, or on the neuromuscular plate. However, this is unlikely, as the ulnar nerve at that level consists only of myelinated sensory and motor fibres, and the absence of synapses in that area that would require transmitters. Additionally, there is no evidence that tizanidine has a direct effect on the nerves. Since imidazolidine substances related to clonidine are thought to inhibit transmitter release, it might be that they inhibit the release of the stored acetylcholine in the presynaptic vesicles in the neuromuscular plate.

Tizanidine reduces spasticity, possibly by increasing presynaptic inhibition of motor neurones. Tizanidine was shown to have no direct effect on the skeletal muscle fibers or the neuromuscular junction. Although having no major effect on monosynaptic spinal reflexes, it has its main effect on polysynaptic pathways. Consequently, it is thought to decrease the facilitation of spinal motor neurons [3]. Muscle reflexes are particularly depressed (polysynaptic reflexes) by tizanidine, which results in both spinal and supraspinal effects. Nevertheless, the exact mechanism of tizanidine action remains unknown. There are clinical studies evaluating a decrease in blood pressure, sedation and sympatholytic effects caused by tizanidine. In addition, in animal studies, tizanidine was shown to have anticonvulsant effects and to inhibit gastrointestinal motility. Tizanidine may have a gastric protective effect due to its acid inhibitory effect through the nervus vagus with noredrenergic suppression [28]. In studies investigating the antispastic effect of tizanidine, it was shown that the frequency of spasm and clonus improved significantly. Thus, tizanidine is primarily effective presynaptically [29–31]. In contrast, the depressant effect of tizanidine on interneurone polysynaptic excitation is due to the reduction of their postsynaptic effects rather than the inhibition of their presynaptic release of excitatory transmitters [32]. However, it is unlikely that these effects directly affected the results of our study, where supramaximal stimulation was administered locally and peripherally by the TOF-Guard device. Animal models also showed that tizanidine has a hypothermic effect [33, 34]. A decreased oxygen consumption and energy expenditure were observed after a single dose of 6 mg and 12 mg tizanidine in healthy subjects [35]. The reduction in oxygen consumption and energy expenditure, and hence the possibility of hastening hypothermia could, theoretically, be vital, since hypothermia is allowed during neuromuscular blockade. In terms of our use of atropine, which is parasympatholytic, it was thought to have no effect in

our study, as it was administered to both groups. Diazepam shows a muscle relaxant effect, and with 5 mg diazepam given at night, active metabloites may have been present in the course of the study. Nonetheless, a significantly longer duration in the muscle blockade was found in the tizanidine group.

Conclusion

In this study, the effect of oral tizanidine on the duration of vecuronium maintenance dose in general anaesthesia was investigated. It was shown that pre-operative tizanidine increased the duration of vecuronium maintenance doses more significantly than placebo. Haemodynamic changes in both groups were not significant. Thus, it is suggested that various doses of tizanidine and various times of prescription prior to operation should be studied. In conclusion, more evidence is needed on the concomitant use of tizanidine and neuromuscular nondepolarizing blockers.

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ORIGINAL PAPER

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First signs of reverse cardiac remodeling following one-month, low dose add-on sacubitril-valsartan therapy in patients with advanced systolic heart failure

Wioletta Sacharczuk
2nd Department of Cardiology, Poznan University of Medical Sciences, Poland
https://orcid.org/0000-0001-6586-7121
Corresponding author: wioletta.sacharczuk@wp.pl

Rafał Dankowski
2nd Department of Cardiology, Poznan University of Medical Sciences, Poznan, Poland
https://orcid.org/0000-0003-0843-5378

Anna Sowińska
Poznan University of Medical Sciences, Poland
https://orcid.org/0000-0001-5319-5057

Artur Baszko
2nd Department of Cardiology, Poznan University of Medical Sciences, Poznan, Poland
https://orcid.org/0000-0002-6064-6458

Andrzej Szyszka
2nd Department of Cardiology, Poznan University of Medical Sciences, Poznan, Poland
https://orcid.org/0000-0003-0471-7001

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ABSTRACT

Introduction. We investigated the early signs of reverse cardiac remodeling in symptomatic patients with heart failure (HF) with reduced left ventricular ejection fraction (LVEF) during one-month, low dose add-on sacubitril/valsartan (S/V) therapy.

Material and Methods. Thirty-seven adult patients with HF were evaluated before and after one-month treatment with a low dose (24/26 mg BID) of S/V.

Results. The patients' mean age was 64.50 ± 17.50 years and median LVEF 29.10%. The S/V treatment resulted in a significant decrease in blood levels of the N-terminal pro-B-type natriuretic peptide (-364 pg/mL; $p = 0.01$), left ventricular end-diastolic (-2 mm; $p < 0.01$) and end-systolic diameters (-2.4 mm; $p = 0.01$), end-diastolic (-9 ml; $p < 0.01$) and end-systolic volumes (-6 ml; $p < 0.01$), the indexed left atrial volume (-8 ml/m^2; $p < 0.01$), effective orifice area mitral regurgitation (-0.09 cm^2; $p = 0.03$). The left ventricular ejection fraction did not change in the course of the study.

Conclusion. One-month, low dose add-on S/V therapy in patients with HF and reduced LVEF induces reverse cardiac remodeling. The long-term effects of a low dose S/V add-on therapy in this group of patients requires further research.

Introduction

Despite the improvement in the management, heart failure (HF) remains a clinically significant problem. The 10-year survival rate is estimated in approximately 30% of patients, compared to 75% in the general population [1]. Optimal therapy is crucial in treating HF patients, as it reduces mortality and hospitalization, improves the quality of life in the symptomatic individuals [2]. Sacubitril/valsartan (S/V) therapy, as an add-on to an already existing pharmacological treatment, improves left ventricular systolic function, exercise capacity, and quality of life in HF patients [3]. During S/V therapy, the beneficial changes in the cardiac structure and function (reverse cardiac remodeling, CRR) are followed by a decrease in N-terminal pro-B-type natriuretic peptide (NT-proBNP) and troponins concentration [4].

Several echocardiographic parameters, such as dimensions or volumes of the left ventricle (LV), left ventricular ejection fraction (LVEF) constitute valuable indicators of CRR [5–7].

Up to date, the studies evaluating S/V-induced CRR analyzed the effect of its full-dose treatment, i.e., 97/103 mg BID after several months of follow-up [8, 9]. However, the target doses are not often achieved in real life, and approximately two-thirds of patients remain on the lowest dose following six months of S/V treatment [10]. No data are available with regard to the early effects of a low dose of 24/26 mg of S/V on CRR of the HF patients. Therefore, we aimed to evaluate the effects of one-month therapy with a low dose of S/V (24/26 mg BID) on the echocardiographic parameters of CRR in individuals with HF with reduced LVEF <40% (HFrEF).

Material and Methods

Study population

Between November 2018 and April 2021, we prospectively enrolled 45 patients with symptomatic HFrEF. The addditional inclusion criteria included NYHA functional class II or III, at least one hospitalization due to heart failure decompensation within the last 12 months, and no previous use of S/V. All patients had to be on an optimal HF therapy recommended by the European Society of Cardiology (2016) and the American College of Cardiology/American Heart Association (2017), which included: beta-blocker and/or ivabradine, angiotensin-converting enzyme inhibitor (ACEI), or angiotensin receptor blocker (ARB), mineralocorticoid receptor antagonist (MRA), and at least one diuretic [1]. The exclusion criteria comprised: myocardial infarction or revascularization within the preceding three months; cardiac resynchronization device implantation within the preceding six months; previous intolerance to ACEI/ARB; symptomatic hypotension; history of angioedema; estimated glomerular filtration rate (eGFR) <30 mL/min/m^2; potassium concentration > 5.2 mmol/L; and poor quality of the transthoracic echocardiographic image. The summary of the study flow is shown in **Figure 1**. The study protocol and the informed consent were in accordance with the Helsinki Declaration and were approved by the Bioethics Committee of Poznan University of Medical Sciences.

Following the enrollment procedure and 36-hours of ACEI wash-out, the patients were started a low dose of S/V, i.e., 24/26 mg BID [11]. During the follow-up period, pharmacotherapy up-titration was not allowed. The NT-proBNP serum concentration and transthoracic echocardiography were performed at the baseline and after one month follow-up.

Echocardiography

During the standard transthoracic echocardiography (Vivid-9, General Electric Medical Systems, USA), cine loops of three cardiac cycles were recorded for the offline analysis with an average frame rate of 56–92 frames/sec. Left atrial and ventricular volumes and diameters were measured according to the American Society of Echocardiography [12]. LVEF was calculated by the Simpson's method from end-systolic and end-diastolic endocardial borders using the apical 4- and 2- chamber views. If two or more contiguous LV endocardial segments were poorly visualized, LVEF was not assessed. Thus, eight patients were excluded from the study, and finally, thirty-seven patients were enrolled in the study.

Statistical analysis

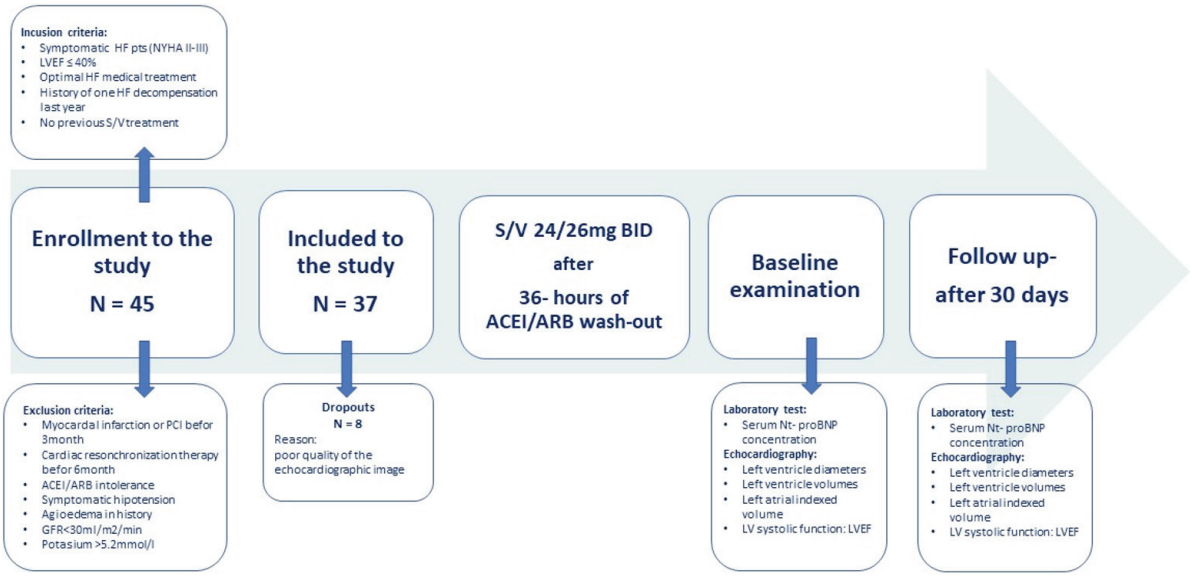
The distribution of the majority of continuous data was not normal according to the Shapiro-Wilk test, hence, the results are presented as medians. The paired nonparametric Wilcoxon

test was used to compare the data before and after one-month treatment with S/V. The p-value less than < 0.05 was considered statistically significant. Data were analyzed using Dell Statistica (data analysis software system), version 13, Dell Inc. (2016).

Results

All patients (mean age 67 years, five females) completed a one-month follow-up. The etiology of HF was ischemic in 23 patients (62%). Twenty-one patients were in NYHA class I-II, and 16 in NYHA class III-IV. The majority of the group (24 patients, 65%) presented with a very severe left ventricular systolic dysfunction (LVEF median 29.10%). The baseline characteristics of the studied group are present in **Table 1**.

After a month, NT-proBNP concentrations were significantly lower (-364 pg/mL, p = 0.01). **Table 2** presents the results of NT-proBNP in the course of the study. We observed a significant reduction of the left ventricular end-diastolic diameter (-2



Abbreviation: ACEI - angiotensin-converting enzyme inhibitor, ARB - angiotensin receptor blocker, GFR - glomerular filtration rate, HF - heart failure, LVEF - left ventricular ejection fraction, S/V - sacubitril/valsartan.

Figure 1. Study flow

Table 1. Patients characteristics at the baseline (n = 37)

	No.	(%)
Gender: – Male/female	32/5	86/14
Etiology: – Non-ischemic – Ischemic	14 23	38 62
Diabetes	16	43
Chronic renal failure (GFR≤ 90 mL per minute)	12	32
Atrial fibrillation: persistent or permanent	8	22
NYHA Class: – I-II – III-IV	16 9	64 36
CRT	8	21
Beta-blocker	36	97
Ivabradine	3	8
Loop diuretic	37	100
Mineralocorticoid-receptor antagonists	32	86
Angiotensin-converting enzyme inhibitor before S/V treatment	37	100

Abbreviations: BMI – body mass index, CRT – cardiac resynchronization therapy, GFR-glomerular filtration rate, NYHA – New York Heart Association Classification.

Table 2. Clinical, laboratory, and echocardiographic parameters at the baseline and at the follow-up (n = 37)

Parameter	Baseline [median value]	Follow-up [median value]	Paired differences	
			[median value]	p-value
SBP (mmHg)	120	120	-3	0.09
DBP (mmHg)	76	74	-1	0.36
NT-proBNP (pg/mL)	1475	1581	-364	0.01
LVEDD (mm)	69	68	-2	<0.01
LVESD (mm)	61.10	59	-2.40	<0.01
LVEDV (mL)	185	169	-9	<0.01
LVESV (mL)	123	112	-6	<0.01
LAVI (mL/m^2)	52	40	-8	<0.01
LVEF (%)	29.10	30.00	0.90	0.07
EROA (mm^2)	0.24	0.15	0.09	0.03

Abbreviations: DBP – diastolic blood pressure, EROA – effective regurgitant orifice area, LAVI – left atrial volume index, LVEF – left ventricular ejection fraction, LVEDD – left ventricular end-diastolic diameter, LVEDV – left ventricular end-diastolic volume, LVESD – left ventricular end-systolic diameter, LVESV – left ventricular end-systolic volume, NT-proBNP – N-terminal pro-b-type natriuretic, SBP – systolic blood pressure.

mm; p < 0.01), left ventricular end-systolic diameter (-2.40 mm; p < 0.01), left ventricular end-diastolic volume (-9 mL; p < 0.01) and left ventricular end-systolic volume (-6 ml; p < 0.01), indexed left atrial volume (-8 mL/m^2; p < 0.01) and the effective regurgitant orifice area of the mitral valve (-0.09 mm^2; p = 0.03). However, the left ventricular ejection fraction did not change significantly during the treatment (LVEF = 29.10 vs 30.00%, p = 0.07). **Table 2** shows the laboratory and echocardiographic parameters during the study.

Discussion

We demonstrated that one-month low dose of S/V treatment in patients with HFrEF results in a significant reverse cardiac remodeling. Furthermore, we observed a small, but significant, reduction of the left ventricular end-diastolic diameter, left ventricular end-systolic diameter, left ventricular end-diastolic volume, and left ventricular end-systolic volume, indexed left atrial volume and mitral regurgitation severity assessed by the effective regurgitant orifice area.

Historically, reverse cardiac remodeling was defined as a 20% decrease in the left ventricular end-diastolic volume [13]. Nowadays, a ≥10% left ventricular end-systolic volume decrease is preferable, since it leads to more favorable outcomes after optimizing HF treatment [14] and strongly correlates with the patients' survival rate [13, 15]. In our study, left ventricular volume (end-diastolic and end-systolic) improved by approximately 9%. It is worth bearing in mind that this effect resulted from one-month, low-dose S/V therapy.

Significant left atrial reverse remodeling is defined as a decrease of 15% of left atrial volume [16]. Despite scarce data, left atrial reverse remodeling may result from pharmacological intervention (data for ACEI or ARB, spironolactone therapy), invasive therapy (e.g., ablation of the pulmonary veins in AF, cardiac resynchronization therapy), or the improvement of diastolic and systolic left ventricle function. Regardless of the mechanism of left atrial remodeling reverse, the left atrial volume significantly influences the prognosis of patients with HF [16–18]. Our study demonstrated that the indexed left atrial volume reduction following one month of low dose S/V therapy is one of the most efficient parameters of reverse remodeling. The severity of mitral regurgitation serves as an independent risk factor for cardiovascular morbidity and mortality [18], therefore, the improvement of left ventricular and atrial volumes reduces functional mitral regurgitation [18], which was also found in our study. All the above-mentioned improvements present completely left-sided reverse cardiac remodeling. Nevertheless, the lack of a significant improvement in LVEF in our study requires some comment. In fact, a specific, layered structure of the myocardium may account for it. The LVEF is more dependent on the function of the midwall circumferential fibers. Thus, better myocardial remodeling requires a long-term treatment in order to observe the improvement in the left ventricu-

lar systolic function, as measured by the ejection fraction [19]. In fact, this may be the reason why Mazzetti et al. observed the LVEF improvement following six months of S/V treatment, and not after three months [19]. On the other hand, the small sample size and the short observation period in our study limit the possibility of drawing definitive conclusions.

On the basis of the latest data, S/V-induced reverse heart remodeling may stem from hemodynamic changes. S/V has reduced cardiac wall stress, thus, decreasing intracardiac pressures [20], which is indirectly demonstrated by the decreasing NT pro-BNP concentration in the blood serum. In our study, the median drop of the NT-proBNP was > 364 pg/mL. This result in in line with the PROVE-HF study, which proved that NT-pro BNP reduction is related to reverse cardiac remodeling [21]. It is worth noting that in the PROVE-HF study, CRR parameters were assessed at the baseline and after 6 and 12 months. In another study, by Januzzi et al., the concentration of NT-proBNP decreased as early as 14 days after the initiation of S/V [22]. In this study, the starting dose of S/V was 24/26 mg BID, and it was titrated after 2–4 weeks to the maximum dose of 97/103 mg BID (65.0% of patients at the end of the study) or maximum tolerated dose.

Additionally, S/V possibly directly affects the concentration of natriuretic peptides through its pharmacological effect on neprilysin, in addition to the impact on intracardiac filling pressures [23]. However, further studies on larger groups are necessary to quantify this effect.

We used a low dose of S/V, i.e., 24/26 mg BID, also referred to as an initial dose in the "conservative high-dose protocol" of the TITRATION study [11]. In this study, S/V was titrated after 2 weeks to 49/53mg BID, and subsequently to a maximal dose of 97/103mg BID following 6 weeks. If a condensed regimen was chosen (a second arm of the study), patients received S/V at the dose of 97/103 mg BID after 2 weeks. The TITRATION study reported the clinical outcomes after at least three months of the S/V treatment (at this point, 85.2% of patients were on S/V 97/103mg BID). In contrast, in our study, a low S/V dose initiates a reverse cardiac remodeling after a month of therapy. According to the current ESC guidelines for diagnosing and treating acute and chronic HF, the recommended target dose of S/V is 97/103 mg BID [2, 24]. However, this dose is not often achieved in real life. In fact, up to two-thirds of patients remain on the lowest dose after six months since the onset of the therapy [25]. In the PARADIGM-HF, in 17.8% of patients a reduction of the S/V dose from 97/103 to 47/53mg BID was necessary. Moreover, in 12% of patients, S/V was withdrawn, primarily due to hyperkalemia or hypotension [3]. In our study, none of the patients required discontinuation of therapy.

Limitations

The main limitation of the study is the small number of participants. However, it was a single-center and unsponsored study including the carefully selected symptomatic patients with advanced HFrEF. Another limitation is the short-term follow-up of one month following the applied treatment. Therefore, we cannot conclusively determine the long-term effects of a low dose S/V. Nevertheless, we demonstrated that this form of treatment exerts a relevant and beneficial S/V effects on the cardiac remodeling in patients with HFrEF. Furthermore, from the economic perspective, it results in reduced costs for the health care system.

Perspectives

We have demonstrated that a low dose S/V for the period of one month reverses adverse cardiac remodeling in the patients with severe HFrEF. Nevertheless, it remains uncertain whether the beneficial effects of low doses of S/V extend beyond one month, and thus should be also investigated. Our HFrEF patients presented with a very severe systolic dysfunction with LVEF 29.10%. Therefore, further research is necessary to investigate whether a low dose S/V would provide similar benefits in patients with a less severe impairment of the systolic function.

Conclusion

One-month S/V therapy with 24/26 mg BID in patients with HFrEF induces the left-sided cardiac reverse remodeling.

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Conflict of interest statement
The authors declare that Novartis did not participate (including financial, content-related, organizational) in the scope of the presented scientific research. Wioletta Sacharczuk, Rafał Dankowski, and Andrzej Szyszka received lecture fees from Novartis.

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Data availability statement
The data supporting this study are available on a reasonable request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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REVIEW PAPER

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Pulmonary embolism in patients with the Coronavirus Disease 2019

Przemysław Kapała
Department of Cardiology-Intensive Therapy, Poznan University of Medical Sciences, Poland
https://orcid.org/0000-0001-7530-439X

Mattia Versace
Università degli Studi di Firenze, Emergency Medicine Residency Program, Florence, Italy
USCA Firenze, COVID-19 Special Unit, USL Toscana Centro, Florence, Italy
–

Ewelina Tuchendler
The Jerzy Gromkowski District Specialized Hospital, The Second Ward of Infectious Diseases, Wroclaw, Poland
–

Anna Marciniak
St George's University Hospitals NHS Foundation Trust, Cardiology Department, London, United Kingdom
https://orcid.org/0000-0001-6590-1780

Przemysław Guzik
Department of Cardiology-Intensive Therapy, Poznan University of Medical Sciences, Poland
https://orcid.org/0000-0001-9052-5027

Corresponding author: pguzik@ptkardio.pl

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ABSTRACT

Coronaviruses are RNA viruses causing infectious diseases. They had been responsible for 15% cases of a common cold before December 2019. With the new strain of coronavirus SARS CoV2 which causes COVID-19 disease, the ongoing pandemic surprised with the severity of symptoms and its course compared to the previously known mild respiratory tract infections. In the end of December 2021, over 274 million people were diagnosed with COVID-19 disease, and the total mortality amounted to nearly 5.4 million deaths in more than 200 countries. One of the potentially fatal complications of COVID-19 is pulmonary embolism (PE). It appears that PE has been associated with several coagulation abnormalities as well as with frequent significantly elevated concentration of D-dimer's. A higher D-dimer concentration in blood serum, in turn, has been associated with an increased risk of premature death. Moreover, inflammation, typical in the course of COVID-19, is considered a prothrombotic condition; higher interleukin 6 (Il-6) and C-reactive protein concentrations have been found in patients with more severe forms of COVID-19. So far, none specific for COVID-19 studies have been available with regard to the diagnosis and treatment of PE. Therefore, the practical approach is based on the experience of other groups of patients. Prevention of thrombotic events seems reasonable, at least in COVID-19 patients with the risk factors of developing venous thromboembolism. Low-molecular-weight heparins are most commonly prescribed (e.g. enoxaparin, dalteparin). Following the confirmed definite PE diagnosis, proper anticoagulation or, if necessary, thrombolytic treatment must be introduced.

Coronaviruses are RNA viruses causing infectious diseases in birds and mammals, including people [1]. However, infections caused by these viruses may be asymptomatic. It usually presents as mild symptoms similar to a common cold, although patients frequently end up developing severe complications which may lead to death [1–5].

For many years, four coronaviruses, HCoV-OC43, HCoV-HKU1, HCoV-229E, and HCoV-NL63, have been present in human populations, and have been responsible for up to 15% of all cases of a common cold [6]. Three other coronaviruses identified in humans, i.e. MERS-CoV, SARS-CoV, and SARS CoV-2, account for more severe infections, i.e., the Middle East severe acute Respiratory Syndrome, Severe Acute Respiratory Syndrome, and the Coronavirus Disease 2019 (COVID-19), respectively [7, 8]. In the end of December 2021, over 274 million people were diagnosed with COVID-19, and the total mortality amounted to nearly 5.4 million deaths in more than 200 countries worldwide [9]. The beginning of the COVID-19 vaccination process in late 2020 provided the humanity with the expected efficient remedy. To date, although over 4400 million people globally have been vaccinated with at least one dose, it is still not enough to gain herd immunity [10].

The most common clinical symptoms of COVID-19 include fever, cough, fatigue, dyspnoea, accompanied with muscle pain, loss of smell and taste, diarrhoea, and weight loss [11–14]. Additionally, some patients also report nausea, emesis, and abdominal pain. The mean incubation time of the virus varies from 4.6 to 6.5 days [15]. It is crucial to bear in mind that the disease may progress to viral pneumonia, acute respiratory distress syndrome, multi-organ failure, and cytokine storm, which leads to death. Major exacerbation of the disease frequently occurs late, over two week following the infection [16]. Furthermore, COVID-19 affects various organs and systems. This, in turn, may lead to serious complications which are not as common or do not occur at all in case of infections caused by rhinoviruses, influenza viruses, adenoviruses, or older coronaviruses [12, 18].

Crucially, COVID-19 is associated with an increased risk of mortality. Among several serious consequences, pulmonary embolism constitutes one of the most common potentially life-threatening complications in COVID-19 patients [18–20], and the underlying causes are numerous. Similarly to other viral infections, COVID-19 is accompanied with an inflammatory response of varying severity, from mild to extreme. However, COVID-19 may be complicated by a cytokine storm along with several coagulation abnormalities, ranging from excessive bleeding to disseminated intravascular coagulation [21, 22].

In COVID-19, the coagulation is activated through several procoagulant mechanisms, including an increase in fibrinogen concentration, activation of platelets and complement pathway, as well as endothelial damage [23, 24, 26]. Interestingly, Han et al. noticed that D-dimer concentration is generally higher in COVID-19 patients than in the healthy individuals [25]. Individuals with a more severe COVID-19 course present higher D-dimer concentration than those with the mild forms [26–29]. Furthermore, other studies also indicate a positive correlation between the D-dimer concentration on admission, morbidity, and mortality rates [30, 31]. In fact, patients with higher D-dimer and fibrinogen-derived peptides concentrations, longer prothrombin time, and activated partial thromboplastin time were at an increased risk of death. Among those who died, a disseminated intravascular coagulation was found in 71.4% compared to 0.6% in the survivors. Several studies aimed to investigate the mechanisms and pathophysiology of the phenomenon referred to as COVID-19 associated coagulopathy (CAC). **Table 1** summarises the coagulation abnormalities found in COVID-19 patients.

Other causes for a higher prevalence of PE in COVID-19 patients include inflammation, immobilization, the coexistence of prothrombotic diseases, and pharmacological therapy. It is crucial to bear in mind that inflammation is considered a prothrombotic condition. Thus, higher concentrations of interleukin 6 (Il-6) and C-reactive protein are typical in COVID-19 patients [28]. Additionally, critically ill patients frequently develop sepsis and septic shock, both of which may lead to a disseminated intravascular coagulation [23], whereas common respiratory and urinary tract infections also temporarily increase the risk of PE [32].

Individuals with symptomatic COVID-19 show poorer exercise tolerance due to fatigue as well as dyspnoea, and they are hospitalized or subject to quarantine. Consequently, patients are either

Table 1. Changes in blood coagulation parameters in COVID-19 patients [25–28]

Changes in blood coagulation	COVID-19 patients vs. healthy individuals
APTT	Longer
PT	Longer
D-Dimer concentration	Higher
INR	Higher
FDP concentration	Higher
Fibrinogen concentration	Higher
Anti-Thrombin III concentration	Lower

Abbreviations: APTT – activated partial thromboplastin time, FDP – Fibrin degradation products, INR – International normalized ratio, PT – prothrombin time.

immobilized, or have a dramatically limited physical activity, which in itself is associated with an increased risk of thromboembolism. Moreover, numerous patients with a more severe COVID-19 course report other prothrombotic comorbidities, such as obesity, smoking, diabetes [33], advanced age, cancer, or prostate hypertrophy in men. Another risk factor for thromboembolism is a prolonged fever which is frequently accompanied by dehydration, as well as the fact that COVID-19 patients with a more advanced respiratory tract involvement require steroid treatment which also has a prothrombotic effect.

Increased numbers of deep vein thrombosis and life-threatening PE have been observed in COVID-19 patients (**Table 2**). Poissy et al. reported a surprisingly high PE number in 107 patients at the Intensive Care Units in Lille University Hospital in France [34], where in comparison with the pre-COVID19 period, the rate of PE was two-fold higher. Furthermore, PE prevalence was twice as high in COVID-19 patients than in individuals presenting with influenza, who were admitted to the same unit in 2019, whereas PE symptoms usually developed during the 6th day of the Intensive Care Unit hospitalization [34]. Grillet et al. [35] diagnosed acute PE in 23 out of 100 COVID-19 patients who presented with severe clinical signs and symptoms, such as an increased respiratory rate, decreased blood oxygen saturation, temperature > 40°C, the presence of other comorbidities, and a need of mechanical ventilation. According to their study, PE was diagnosed within the first 12 days of the onset of symptomatic COVID-19 infection. In fact, observations from other countries confirmed these findings, e.g. Poyiadji et al. [36] have shown that 72 out of 328 patients diagnosed with COVID-19 also developed PE. Nevertheless, most patients from this study did not require admission to the Intensive Care Unit.

The diagnosis of PE in COVID-19 patients constitutes a challenge, since most clinical signs and symptoms are non-specific for acute PE. A number of the clinical findings present in PE are also frequently found in COVID-19 patients who do not develop PE, i.e. both COVID-19 and PE patients complain of dyspnoea, reduced exercise tolerance, tiredness, and some with chest pain and reduced blood oxygen saturation. Additionally, the D-dimer concentration is increased in both COVID-19 and PE patients. Therefore, the principles or prognostic scores usually applied for the

Table 2. Overview of the studies regarding pulmonary embolism prevalence in COVID-19

	Country	Number of patients	Diagnosed PE	% of PE
Poissy et al. [34]	France	107	22	20.6
Grillet et al. [35]	France	100	23	23
Poyiadji et al. [36]	USA	328	72	21.9
Helms et al. [37]	France	150	25	16.7
Lodigiani et al. [38]	Italy	61	2	3.6
Klok et al. [39]	Denmark	184	65	35
Thomas et al. [40]	Great Britain	63	5	7.9
Middeldorp et al. [41]	The Netherlands	198	11	5.6

Abbreviations: PE – pulmonary embolism.

diagnosis of PE should also be used in COVID-19 patients, and up to date, no distinct or better approaches have been proposed. **Table 3** includes the prognostic scale for PE, and **Tables 4** and **5** summarises the two scores for PE prediction.

On the basis of the available literature and the analysis of three different COVID-19 patients from our centres, we have summarised the values of the Wells' score, the Revised Geneva Score, as well as PESI, and sPESI scores.

It is noteworthy that according to the Wells' score, 7 out of 12 COVID 19 patients with confirmed PE presented a low probability of PE (**Table 6**). In contrast, according to the Geneva Score, 5 of these patients should be categorized as belonging to the low PE probability group. Moreover, neither of the scales classified any of the patients as a high probability of PE. However, prior observations suggest that prognostic scales for PE are not sensitive and specific enough

Table 3. The Pulmonary Embolism Severity Index (PESI) and simplified PESI (sPESI) prognostic score for pulmonary embolism

	PESI	sPESI
Age	Age in years	1 (if >80)
Sex	Male (10)	-
Neoplasm present or not	30	1
Heart failure	10	1
COPD	10	1
Heart Rate >110/min	20	1
Respiratory Rate >30/min	20	-
Systolic Blood Pressure <100	30	1
Temperature <36	20	-
Confusion	60	-
Blood oxygen saturation <90%	20	1

Scoring: (risk)	PESI	sPESI
Very low <65	0–1.6%	0–1.0% risk of death
Low 66–85	1.7–3.5%	>1–10.9% risk of death
Moderate 86–105	3.2–7.1%	
High 106–125	4–11.4%	
Markedly high >125	10–24.5%	

Abbreviations: COPD – chronic obstructive pulmonary disease

Table 4. The Wells' score for the prediction of pulmonary embolism

	Wells (Original)	Wells (Simple)
Previous PE or DVT	1.5	1
Immobilisation for at least three days, or a surgery in the four previous weeks	1.5	1
Malignancy	1	1
Haemoptysis	1	1
Heart Rate > 100 beats/min	1.5	1
Clinical signs of DVT	3	1
Alternative diagnosis less likely than PE	3	1

Interpretation (Original Scale):	
>6 points	High probability
2–6 points	Moderate probability
<2 points	Low probability
Interpretation (Simple):	
≥2 points	Probably PE
<2 points	PE unlikely

Abbreviations: DVT – deep vein thrombosis; PE - pulmonary embolism.

Table 5. The Revised Geneva Score for the pre-test probability of pulmonary embolism

Revised Geneva Score	Points
Previous PE or DVT	3
Surgery (under general anaesthesia) or the lower limb fracture in the past month	2
Malignancy	2
Haemoptysis	2
Age >65	1
Unilateral lower limb pain	3
Pain on deep palpation of the lower limb and unilateral oedema	4
Heart Rate 75–94 beats/min	3
>95	5

Interpretation:	
0–3 points	Low probability
4–10 points	Moderate probability
11 and more points	High probability

Abbreviations: DVT – deep vein thrombosis; PE – pulmonary embolism.

Table 6. Pulmonary embolisms in COVID-19 patients – collected cases and our data

Country	Gender	Age	Comorbidities	Wells scale	Geneva scale (revised)	sPESI	PESI
Abington, USA [42]	F	59	Hypertension, diabetes	1.5	5	2	99
London, UK [43]	M	53	none	1	5	0	63
San Diego, USA [44]	M	42	none	1	2	0	72
Lugano, Switzerland [45]	M	50	chronic kidney disease, hypertension, hepatitis B	2	5	1	80
Manchester, UK [46]	M	52	none	1	0	1	62
Birmingham, UK [47]	F	52	Obesity, diabetes	2	5	3	202
Istanbul, Turkey [48]	F	41	Diabetes	1	0	3	161
New York, USA [49]	M	38	Obesity	2	5	2	118
Poznan, Poland	F	39	Hypertension	3	5	0	39
Florence, Italy	M	64	Previous myocardial infarction, allergic asthma	1.5	3	0	74
Wroclaw, Poland	F	69	None	1	0	0	89
London, UK	M	48	Obesity	2	5	2	118

[50]. The abovementioned data indicate that the active COVID-19 should probably be included in the modified Wells' scale and Geneva Score in order to improve their applicability in this particular group of patients. Therefore, to achieve this a large multicentre registry of patients with COVID-19 who developed PE and who did not develop PE should she established.

Although there are several different PE diagnosis modalities, in COVID-19 patients probably the most frequently applied method is computed tomographic pulmonary angiography. According to Sabri et al. [51], chest CT shows ground-glass opacities and consolidations in most COVID-19 patients (93.4% and 92.6%, respectively). In addition, nearly two-thirds (63.5%) of patients presented the peripheral distribution of lung abnormalities and some central and diffuse opacities (14.3% and 17.5%, respectively). In fact, compared with the reverse-transcription polymerase chain reaction (RT-PCR), chest CT may constitute a more reliable, practical, and faster method to diagnose and assess COVID-19, particularly in the area affected by the epidemic. As Ai, Yang et al. observed, with RT-PCR results as the reference standard in 1014 patients, the sensitivity, specificity, and accuracy of chest CT in indicating COVID-19 infection were 97%, 25% and 68%, respectively [52]. In some centres, lung ultrasound is also employed, particularly at the Emergency Departments or in cases of patients in a critical condition. Moreover, lung point-of-care ultrasound (POCUS) has shown sensitivity and specificity similar to chest CT when diagnosing interstitial pneumonia, which is also an effective method in terms of the visualization of the sub-pleural lung infarctions typical of pulmonary embolism.

It is vital to bear in mind that echocardiography plays an essential role in diagnosing and monitoring patients with suspected PE [53]. In fact, it may directly visualize the thrombus or thrombi within the right ventricle, particularly in patients with the prothrombotic state, such as SARS-COV-2. Echo allows visualizing right ventricular dilatation and dysfunction. It also allows assessing pulmonary artery pressure and elevated pressure features in the form of interventricular septal flattening. The critical part of the echo is the McConnell's sign, characterized by akinesia of mid-segment of RV free wall with a normal apex motion [54]. Although echocardiography may have a low sensitivity in diagnosing PE, at the same time it shows high accuracy in diagnosing a large PE [55]. An essential element, therefore, is thrombotic events prevention which should be introduced to COVID-19 patients with risk factors of developing venous thromboembolism to prevent PE development [56]. Nevertheless, a recent systematic review has pointed out other risk factors of developing directly PE in covid-19 patients [57], such as mechanical ventilation status or parenchymal damage. Interestingly, authors of this study claim that age and typical comorbidities were nor associated with the occurrence of PE.

The most commonly prescribed medications include low-molecular-weight heparins (e.g. enoxaparin, dalteparin), as well as alterna-

tive pharmacological therapies. [58, 59] However, one retrospective study indicated that the use of fondaparinux, instead of low-molecular-weight heparins, should be discouraged [60]. Yet, there is limited evidence that Oral Anticoagulants are ineffective in reducing mortality [61], and apixaban and rivaroxaban concentrations may be increased during the treatment with sarilumab or tocilizumab [56]. Once the definite PE diagnosis is confirmed, an anticoagulation treatment should be initiated, which usually comprises low-molecular-weight heparin or unfractionated heparin [58]. In PE patients who are hemodynamically unstable due to severe and unresponsive hypotonia and/or shock, thrombolysis is recommended with a recombinant fibrin-specific plasminogen activator. Therefore, it is still uncertain which therapy should be eventually chosen for patients with arteriovenous thromboembolism, including those with PE, and further investigations are necessary. Similar considerations apply to the prevention of embolism incidents. Despite these concerns, treatment based on the current guidelines should be introduced in clinical practice and such guidelines have been developed in several countries [58].

Summarizing, COVID-19 infection is associated with multiple coagulation abnormalities leading to the development of PE, particularly in the critically ill patients. PE constitutes one of the most serious complications accompanying COVID-19, as it substantially increases mortality. Hence, the diagnostic approach and the applied therapy should be the same as in non-COVID-19 patients. So far, no specific studies with regard to the diagnosis and treatment of PE in COVID-19 patients have been available. Therefore, it appears that this clinical challenge requires more attention and deserves a prospective clinical evaluation.

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Conflict of interest statement
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REVIEW PAPER

JMS Journal of Medical Science

Polygraph analyses: technical and practical background

Jakub Winkler-Galicki*
Department of Physiology, Poznan University of Medical Sciences, Poland
https://orcid.org/0000-0003-1415-6252
Corresponding author: jwinklergalicki@ump.edu.pl

Joanna Bartkowiak-Wieczorek*
Department of Physiology, Poznan University of Medical Sciences, Poland
https://orcid.org/0000-0002-8347-0512

Dominik Synowiec
Department of Physiology, Poznan University of Medical Sciences, Poland
–

Renata Dąbrowska
Department of Forensic Medicine, Poznan University of Medical Sciences, Poland
https://orcid.org/0000-0003-1575-9653

Edyta Mądry
Department of Physiology, Poznan University of Medical Sciences, Poland
https://orcid.org/0000-0002-0081-6558

* – equal contribution

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ABSTRACT

A lie is not the truth, a common definition found in each dictionary or encyclopaedia. Humans use it in different situations and for various reasons, but in the case of forensics, recruitment, and trust in the company or family, it can be curtailed in order to avoid it or to detect it. One of the possible detection tools is the polygraph, whereby lies may be registered and interpreted by means of physiological activities controlled by the autonomic nervous system, such as sweating, trembling, or changes in breathing or in the heart rate. The analyses of the aforementioned parameters are monitored in response to questions, thus, providing information about a possible lie. Questions should be asked according to one of the approved protocols and given procedures and algorithms, which are constantly developed and revised to provide the best possible results.

Introduction

The lie is one of the most common tools used by humans, and it is thought that it has been present since the beginning of mankind [1]. The conflict generated during lying in the human brain results in an increase in stress expressed on the behavioural level as a fight-or-fly reaction. The physiological manifestations, such as changes in blood pressure, heart rhythm and rate, breath deepness, and skin resistance changes, can be monitored in an attempt to detect a lie. The modern polygraph, an appliance used for recording changes in organ activity during true-false verification, is a small portable device (**Figure 1**). Together with the mobile PC, the polygraph registers the chang-

Figure 1. An example polygraph setup comprising a CPSpro – Stoeltinga and Dell mobile PC

es creating a record of the physiological fluctuations, since stress responses are regulated by the autonomic nervous system [2, 3].

It is necessary to emphasize the external conditions which can affect the examination, such as alcohol, or drugs, as well as the exclusion criteria. Bradley and Ainsworth (1984) [4] found that alcohol intoxication during a sham crime reduces crime detection accuracy, although Hammond (1980) [5] observed no difference in the detection of fraud using a sham crime scenario among healthy individuals, alcoholics, and psychopaths. Similarly, neither Raskin and Hare (1978) [6] nor Patrick and Iacono (1989) [7] found any differences in the detection of fraud between psychopathic and non-psychopathic prisoners. However, the studies of Waid et al. [8] indicate that the use of meprobamate reduces polygraph tests accuracy, although the studies conducted by [9,10] suggest that similar drugs, such as diazepam (Valium) and methylphenidate (Ritalin), show little effect on the polygraph results. Summarising, it is possible to state that drugs and alcohol may impact the result of the analyses, nevertheless, currently, researchers are aware of that and, therefore, are able to extrapolate the effect on the results.

Interestingly, polygraph analyses cannot be used in all cases. According to the American Polygraph Association (APA), the test can be performed only if several criteria are fulfilled:

- a person does not present with any mental disorders,
- the person tested is over 12 years of age and is able to think in an abstract manner,
- the IQ is higher than 55 and mean age equivalence indicates an age of not less than 12 years,
- the person has a Global Assessment of Functioning scale score of less than 50 [16]

Physical disabilities (e.g. amputation of a finger, arm, hand, as well as spine injures) are potentially problematic; however, the sensor can be omitted, or placed elsewhere, for instance, EDA can be placed not on the finger, but on a foot [16].

In practice, polygraph tests constitute valuable tools for detecting truthfulness, confabulation and fraudulent behaviour in various areas (criminal departments, national security agencies, business, industry, science):

- for detecting lies in forensics (criminal investigation) [11],
- as evidence and circumstantial evidence for government agencies and legal systems [12],
- to identify terrorism suspects and other individuals presenting anti-social behaviour who may be concealing their identity [13],
- as a tool supporting the administration of justice to individuals facing wrongful convictions [14],
- in industry and business, they can contribute to increasing the organisational efficiency, facilitating the selection of candidates with appropriate skills and experience, and promoting the employee integrity in the workplace [15].

The physiological parameters can be recorded using different body parts (**Figure 2**), for instance, the chest, arm, and fingers (**Table 1**). For proper analyses, the following procedures should be followed:

1. Pre-test interview.
2. Acquaintance test.
3. Main test.
4. Final talk.
5. Data analyses.

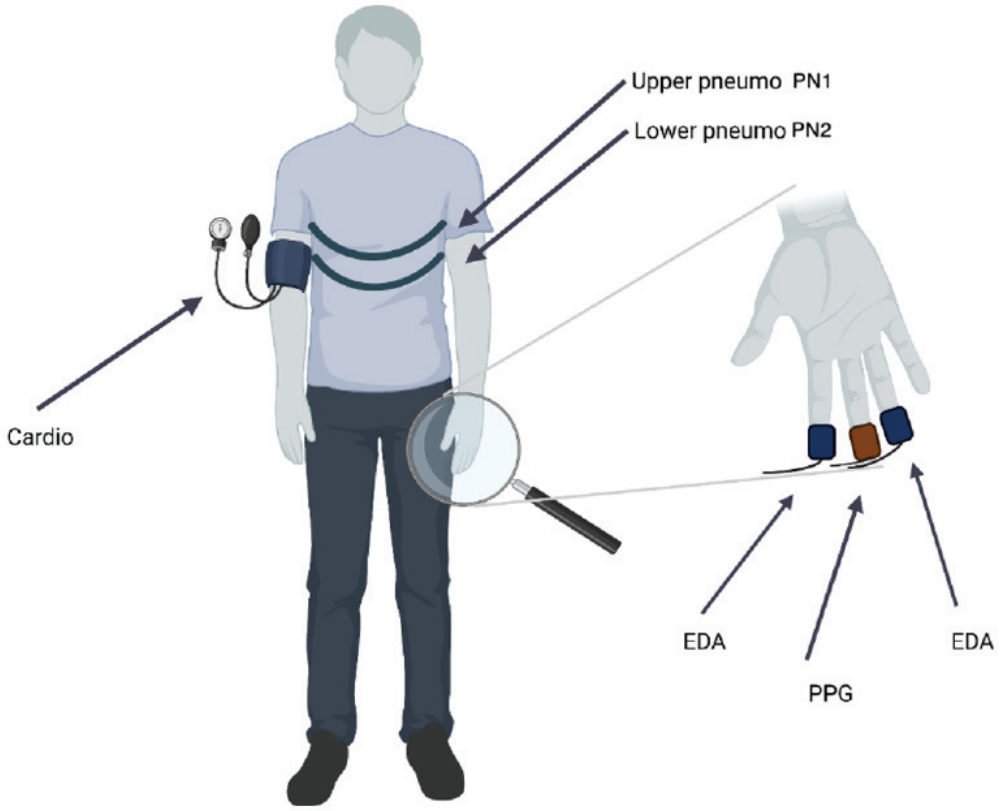

Figure 2. Location of sensors: Upper pneumo PN1 – Thoracic Pneumograph; Lower pneumo PN2 – Abdominal Pneumograph; Cardio – Blood pressure measurement; EDA – Electro Dermal Activity (Galvanometer); PPG – PhotoPlethysmograph (Created with BioRender.com)

Table 1. Physiological parameters and channels used in a polygraph examination [16]

Physiological parameter	Observed change	Type of sensor	Sensor location
Respiratory rhythm	– apnoea – breath suppression – breath rate retardation – breath rate acceleration – shallow breathing – respiratory depression	Pneumo	Upper and lower chest
Skin ectodermal activity	– skin dryness – hyperhidrosis	EDA	Fingers
Blood pressure and heart rate	– heart rate depression – heart rate acceleration – blood pressure fluctuations – extrasystoles	Cardio	Arm or thumb
Blood volume changes in veins	– decreases of the volume in small and distal vessels	PPG / PLE	Tip of the finger

Test procedure

Regardless of the reason for the test (forensic investigation, screening, legal evidence of offenders or private purposes), the examiners have to prepare a suitable place, the equipment as well as themselves. The test should be conducted in quiet and private conditions, in a location with no disturbance or disruption, whereas the examined person needs to understand the language and all the question asked, and crucially, the person cannot be forced, but must express a willingness to be tested [16]. The examiner should have all the available background information concerning the investigated issue so that they can ask pertinent questions as well as select the appropriate and most adapted protocol and set of questions [17].

Pre-test interview

The interview includes the verification of the subject's identity, obtaining written consent to participate in the procedure, explaining the main principles of the polygraph test, a comprehensive discussion of the main issues which will be verified during the test, familiarisation with the test questions and their discussion. The pre-test interview should reduce the participant's stress by asking questions regarding basic personal information, such as occupation, age, health status and memory [18], which also helps to determine if a person is physically and mentally fit to take part in the test. Additionally, the examiner should also explain the procedure and ensure that the participant understands that they will only be asked questions concerning the investigated issue [16]. In this part the examiner frequently asks, such questions as "Do you understand that you will be asked only the questions about the case?" or "Do you understand all the steps in the procedure?" [16].

Acquaintance test

This test is also referred to as a demonstration test (abbreviated as "demo"), and it is designed to relax or activate the participant. In addition, it also reveals the participant's typical level of emotional agitation when responding to answers, which allows to observe the variability of the registered physiological reactions, particularly when the person is deliberately misleading (so-called psychophysiological response to a known lie). Furthermore, the acquaintance test also enables the participant to grow accustomed to the testing situation and the installed sensors [19]. This part of the procedure is based on irrelevant questions (described in „Main test" section).

Main test

The main test is central to the investigation and involves questions pertaining to the issue under investigation asked in 20–25 second intervals to provide time for recovery [16]. The participant should be instructed to provide only "yes" or "no" answers, and the questions should be simple and close to the true/false mode [16]. The questions are the most crucial element of the procedure, since they constitute emotional triggers, eliciting physiological responses which can be detected and recorded for further analysis (**Table 2**). The questions can be categorised as shown in **Figure 3**.

The questions should:

- be simple, direct and easy to understand,
- be determined and adjusted in time,
- describe the relationship between the participant and the issue,
- not lead to incorrect answers,
- aim at complex references,
- avoid sophisticated law, medical, psychological, motivation, terminology, vernacular and jargon,
- be adjusted in terms of complexity [16].

The interview procedure should be based on one of the commonly applicable techniques, such as CQT (Control Questions Test), GKT (Guilty Knowledge Test) or CIT (Concealed Infor-

Table 2. Question types and examples [17]

Question type	Abbreviation	Description	Example
Irrelevant Questions	IQ	The questions are not case-specific, aim to identify the most common reactions during the acquaintance test; serve to eliminate stress and orientate the responses	Is it currently the year 2020? Is Anna your name?
Relevant Questions	RQ	The questions pertain only to the investigated case and its circumstances	Did you steal the jewellery? Did you use a hammer to break the window?
Sacrifice Relevant Questions	ScR	Usually asked as a first relevant question to determine the intention of telling the truth	Are you going to answer the questions concerning the case precisely and accurately?
Control Questions	CQ	The questions address similar situations and cases as that under investigation	Had you stolen anything before turning 18?
Probable Lie Comparisons Questions	PLC	A question in response to which the participant is likely to lie	Did you ever lie in the first 20 years of your life?
Exclusive question	C(ex)	The question which does not overlap the time and/or case under investigation	Have you been to this place during your studies?
Inclusive question	C(in)	The question generally addresses the main case, but does not ask about it directly	Have you ever stolen anything from the shop?

mation Test) and can follow protocols such as the US Federal You-Phase, ZCT (Zone Comparison Test), Utah ZCT (Utah Zone Comparison Test), Utah ZTC DLC (Utah Zone Comparison Test Directed-Lie Test), USAF (United States Air Force) etc. (**Table 3**) [16]. The general principle of each protocol is to randomize the questions and to mix them in order to create the most unique combination, thereby allowing the detection of a lie [16].

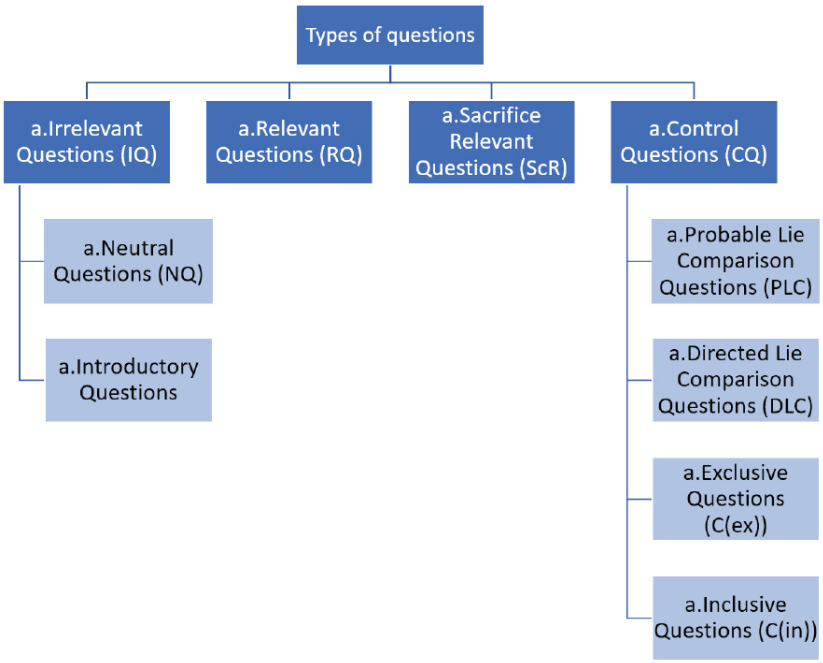

Figure 3. The categorisation of questions

Table 3. The types of questions used in the different protocols [16].

Protocol type	Question series	Question Set
Utah ZTC	Series I	I[1], ScR[2], N1[3], C1[4], R1[5], N2, C2, R2, N3, C3, R3
	Series II	I, ScR, N2, C3, R2, N3, C1, R3, N1, C2, R1
	Series III	I, ScR, N3, C2, R3, N1, C3, R1, N2, C1, R2
Federal ZCT	Series I	N1, ScR, S[6]1, C1, R1, C2, R2, S2, C3, R3
You-Phase (Bi-Zone)	Series I	N1, ScR, S1, C1, R1, C2, R2, C3, S2
USAF MGQT	Series I (option I)	N1, ScR, C1, R1, C2, R2, C3, (R3, C16, R4)
	Series I (option II)	N1, ScR, C1, R1, R2, C2, (R3, C3, R4, R5, C4,)
Utah MGQT	Series I	I, ScR, N1, C1, R1, R2, C2, N2 (optional), R3, R4, C3, N3
DLST (TES)	Series I	N1, N2, ScR, C1, R1, R2, C2, R1, R2, C1, R1, R2, C2
LEPET	Series I	N1, ScR, C1, R1, C2, N2, R2, C3, R3, C4, R4, C5
	Series II	N1, ScR, C1, R1, C2, R2, N2, C3, R3, C4
	Series III (deepen)	N1, ScR, C1, R1, C2, R2, C3, R3, C4, R4, C5
IZCT	Series I	N1, 2I, ScR3, 4N, C(ex)5[7], R6, N7, 8C(in)[8], R9, N10, C11, R12, Cm[9]13

1. Introductory question e.g.: Do you understand that in the test I am going to ask only about the case we have talked about?
2. Sacrificed relevant question e.g.: Are you going to answer the questions concerning the case precisely and accurately?
3. Neutral question e.g., Is it currently the year 2020?
4. Comparison question e.g., Had you stolen anything before turning 18?
5. Relevant question e.g., Did you use a hammer to hit the man?
6. Symptomatic question e.g., Are you afraid that I will ask you about something else than the investigated case, even though I promised that I would only ask you about it?
7. Exclusive question e.g., Did you steal food while studying at the university?
8. Inclusive question e.g., Have you ever stolen anything?

The final talk

The test should be completed with the final talk, during which the participant is not informed about the results, rather reassured about the validity of all the steps.

Data analyses and opinion

The polygraph expert does not provide their evaluation directly after the test since the polygrams need to be assessed taking into consideration the pre-interview results, as well as the personal and case background information. It is crucial, due to the fact that a purposefully non-cooperative (PNC) person may try to restrict their physiological reactions [16]. There are numerous guidelines dedicated to polygraph data analyses. The ones currently used by licensed professionals are based on the scientific, physiological, psychological, neurophysiological and psychophysiological approaches, as well as the decision theory and signals reception theory [16]. On the basis of the physiological parameters, the data collected during the polygraph are taken into account when evaluating the polygrams and assessed based on numerical scales, that is, each relevant question is assigned a specific numerical value. Subsequently, the results obtained for the physiological responses recorded for the relevant questions are compared with the control questions. Each measured physiological parameter is evaluated separately.

The most objective visual analysis method of the test data in polygraphy research is the numerical analysis according to the Empirical Scoring System (ESS). It is used in the comparative questioning techniques (CQT) and the CIT test. The ESS analysis uses a 3-point rating scale in the range: +1, 0, -1 with the exception of the electrodermal activity (EDA) sensor, which is assigned values in the range of: +2, 0, -2 [21–27]. On the basis of the empirical research, the following diagnostic conditions are distinguished for individual recording channels: pneumo, EDA / GSR, cardio, PLE / PPG. The established decision thresholds are applied after numerical scores are assigned to the response records to the critical questions. In turn, test scores are calculated according to the abovementioned scores – in (-1)-0-1 scale for minor sensors and (-2)-0-2 for major, where (-2) means lie, 0 non-deciding and 2 truth answers. Then, after summing up, the percentage of the answers considered to be true can be obtained.

Archiving of results, notes, any created papers and complete documentation of the procedure should be conducted according to the national and international law, e.g. Polish national law and the European Directive (EU) 2016/680 of the European Parliament and the Council of 27th April 2016 [28] protects natural persons concerning the processing of personal data by competent authorities for the prevention, investigation, detection or prosecution of criminal offences or the execution of criminal penalties, and the free movement of

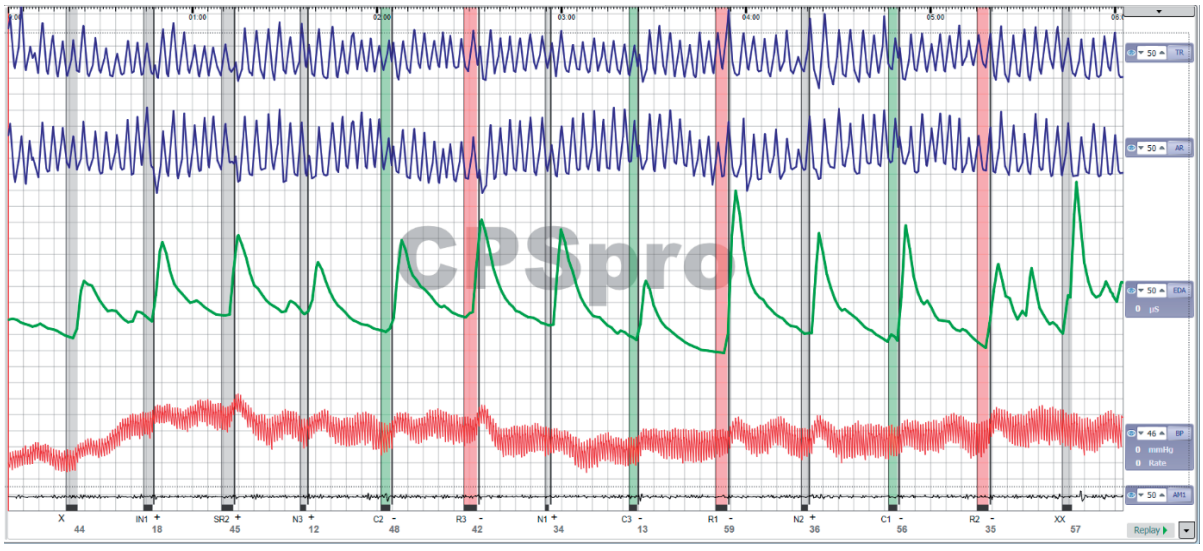

Figure 4. An example of a 5-channel polygram obtained by CPSpro - Stoelting

such data, and the repealing Council Framework Decision 2008/977/JHA.

Conclusions

The polygraph test procedure is based on the preparation of relatively simple questions in a moderately complicated protocol, which are asked in a strictly defined order, with a simultaneous measurement of the physiological reactions and responses in order to detect lies, or establish the objective truth. Polygraph procedures can be used for various purposes in different scientific areas, such as forensics, law, medicine or biotechnology, from finding an error to assessing honesty and truthfulness in the recruitment process, employee validation or a private loyalty manner. Since different polygraph procedures are required depending on the intended application, it is essential to consider the merits according to the polygraph techniques, protocols, validation tests, approaches and the situation. There are different ways to conduct the procedure and it will mainly depend on the issue and the person under investigation, as well as on the expert's preferences. Polygraphic analysis can be used not only to reveal the truth and lies, but they also provide significant insights with regard to stress and memory, due to a thorough analysis of bot the physiological parameters and the action of the autonomic nervous system. In addition, in terms of the diagnostics and personalization of therapy, particularly pharmacotherapy, polygraph tests allow to precisely determine the dosage on the basis of reliable effects of the therapy. It should be emphasized that the application of the polygraph in medicine and science will facilitate the introduction of a completely new quality also in the field of medical questionnaire research. According to our knowledge, the verification of the patient's declared data by means of physiological signals, regulated by the autonomic nervous system, would be a neurophysiological "authorization" of the patient's declaration of reliability. In this context, the polygraph may become a handy tool supporting the credibility of medical data [29–32].

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Conflict of interest statement
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REVIEW PAPER

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Rare occurrence of type 2 diabetes mellitus in patients with sickle cell anaemia: assessing the contribution of inflammation, insulin resistance and glucose buffering capacity of abnormal haemoglobin

Sheu Kadiri Rahamon
Department of Immunology, College of Medicine, University of Ibadan, Nigeria
https://orcid.org/0000-0003-0305-5026
Corresponding author: sk.rahamon@mail1.ui.edu.ng; adekunlesheu@rocketmail.com

Sunday Peter Ogundeji
Department of Haematology, University College Hospital, Ibadan and College of Medicine, University of Ibadan, Nigeria
https://orcid.org/0000-0003-2368-9237

Samson Eshihokhale Olerimi
Department of Biochemistry, Edo University, Iyamho, Nigeria
https://orcid.org/0000-0003-3184-8672

Kehinde Sola Akinlade
Metabolic Research Unit, Department of Chemical Pathology, University College Hospital, Ibadan and College of Medicine, University of Ibadan, Nigeria
https://orcid.org/0000-0002-9413-3562

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ABSTRACT

This review was designed to discuss the rare occurrence of diabetes mellitus (DM) in patients with sickle cell anaemia (SCA) with a particular focus on factors, such as life expectancy, body weight, chronic inflammation, insulin resistance, glucose buffering property of haemoglobin, and microRNAs (miRNAs), aiming to stimulate research which will fill the existing knowledge gaps regarding the interplay between SCA and DM. Additionally, possible pharmacotherapeutic approaches to DM were also highlighted in the review. Google Scholar and PubMed search engines were used to search for the relevant keywords, such as sickle cell trait, sickle cell disease, sickle cell anaemia, insulin resistance, and diabetes mellitus. SCA patients appear to have β-cell dysfunction with a reduced insulin secretion, but present a similar insulin sensitivity status as other patients without haemoglobinopathy. Glucose buffering property of haemoglobin and the possible DM-protective roles of miRNAs in the sickled erythrocytes constitute some of the potential factors protecting SCA patients from developing DM. Sickle cell anaemia is associated with several complications and endocrinopathies, nevertheless, its coexistence with DM continues to be a rare observation. Proper elucidation of the mechanisms which seemingly confer 'protection' against DM in patients with SCA may provide some therapeutic insights regarding DM.

Introduction

Diabetes mellitus (DM) is a group of metabolic diseases with hyperglycaemia as its main feature. Generally, DM can be classified into the following categories; type 1 DM (T1DM), type 2 DM (T2DM), gestational DM (GDM) as well as specific types of DM due to other causes [1]. Of all the types of DM, T2DM constitutes about 90 to 95% with the disease estimated to affect over 350 million people in 2030 [2]. Despite this global epidemic of T2DM, individuals with sickle cell anaemia (SCA) seem to present some form of protection against the disease [3].

Sickle cell disease (SCD) is a Mendelian genetic disease encompassing a wide spectrum of disorders [4]. Its most common form is the homozygous HbS, referred to as sickle cell anaemia (SCA). SCA results from a single nucleotide substitution in the DNA of adenine (A) with thymine (T) at codon 6 of the beta-globin gene on chromosome 11. This substitution causes a point mutation, with hydrophobic valine replacing hydrophilic glutamic acid in the polypeptide of the beta-globin chain of haemoglobin [5–7]. The combination of two normal alpha-globins and two mutant beta-globins forms haemoglobin S (HbS) which polymerizes upon deoxygenation. The monomers aggregate into multiple polymer bundles (rod-like structure) which subsequently lead to red cells deformation from the normal biconcave structures into the sickle shape [6].

Generally, SCA is characterized by haemolytic anaemia, acute and chronic tissue ischaemia (as a result of intermittent occlusion of small vessels) and organ dysfunctions, including endocrine dysfunction/metabolic disorders, such as osteopenia, hypogonadism, carbohydrate intolerance, and primary hypothyroidism [8–12].

Sickle cell anaemia and diabetes mellitus – epidemiology

Despite the established association between SCA and endocrine organs dysfunction, the co-existence of SCA and diabetes mellitus (DM) remains rare [3, 13]. Although the number of people with type 2 DM and sickle cell trait (SCT), a heterozygous form of sickle cell disease, is increasing [14], T2DM rarely develops in individuals with SCA. The infrequent concomitance of the two diseases prompts the belief that SCA may have some protective effects on the development of DM [15]. This protective effect is unexpected as SCA-associated chronic inflammation [16], defective lipid metabolism [17], oxidative stress [18] and endocrinopathies [10] resulting from iron overload (since blood transfusions are a major form of treatment in patients with SCA) are significant harbingers of insulin resistance (IR) and T2DM.

Zhou et al. [19] demonstrated that the prevalence of T2DM in SCD patients is comparable to the prevalence of T2DM among the African-American population in the US. They reported that the unadjusted prevalence rates of T2DM in SCD population of 7,070 adults increased from 9.8% in 2009 to 11.8% in 2014 resulting in a 0.2% – 0.5% year-to-year change; however, when age-and sex-standardized, the prevalence increased from 15.7% in 2009 to 16.5% in 2014. Furthermore, Skinner et al. [20] reported that SCT could increase the risk of development of T2DM-related complications, including retinopathy, nephropathy and hypertension.

Although the report of Zhou et al. [19] indicated an increasing trend in the prevalence of T2DM in SCD patients, no stratification of the glycaemic status was observed, based on the type of SCD, also referred to as SCA (HbSS), or other milder forms of SCD (HbSC, HbSD, HbSE and HbSO). Regardless of this report, the numerous available reports still indicate that the co-existence of T2DM and SCA is uncommon [3, 13]

In 1979, a study by Morrison et al. [21] failed to detect a single case of DM in 711 patients with SCA. In 1987, the co-existence of the two diseases was reported in 2 pregnant women (GDM in SCA patients) [22]. In fact, in 2006, the report from a multi-centre study of Iron Overload demonstrated that DM affects only about 2% of patients with SCA, and that transfusion duration was strongly associated with T2DM [23].

In Nigeria, a survey conducted by Reid et al. [24] failed to identify a single patient suffering from the two diseases simultaneously. However, in the same country in 1990 the first case of SCA and DM co-existence was reported [25]. This was followed, in the same year, by the report of Adekile and Jegende [26] which showed the co-existence of type 1 DM (T1DM) and SCA in a 10-year old child.

These earlier reports were corroborated by a few recent reports documenting the rare co-existence of the two diseases [15, 27–29]. Recently,

Prusty et al. [13] have reported a prevalence of 1.46% in 137 patients with SCA. Similarly, Jang et al. [3] concluded that chances of developing obesity and diabetes over a lifetime in patients with SCD were low. Therefore, the question remains whether it can be argued that SCA is protective against DM. Nevertheless, addressing this issue is currently being investigated through a number of factors which have not been well explored to date.

Underestimation of DM in SCA patients

Glycated haemoglobin (HbA1c), formed when glucose binds specifically to the N-terminal valine of the haemoglobin β chain, is widely used in the screening, diagnosis and monitoring of DM [30]. However, it is well established that HbA1c tests are influenced by conditions affecting both erythrocytes lifespan and by hemoglobinopathies [31, 32]. Thus, discrepancies may occur between HbA1c values and the true clinical situations of the patients [33].

The reliability of HbA1c test is impaired by haemoglobinopathies, as the normal process of non-enzymatic glycation of HbA to HbA1c is impaired. HbA1c estimation using immunoassay and HPLC methods is interfered with by HbS, although it can be measured optimally using enzymatic assays and capillary electrophoresis [33–35]. Alternatively, measurement of non-traditional glucose control markers (albeit their limitations and poor diagnostic guidelines), such as fructosamine, glycated albumin, 1,5-anhydroglucitol, could provide the necessary data [14, 36, 37]. Therefore, it must be taken into consideration that HbA1c measurement alone (depending on the methodology), without blood glucose estimation, may not be sufficient for the diagnosis of pre-diabetes or diabetes in SCA individuals [14, 38]. In fact, the report of Mohamed et al. [15] demonstrated that most studies reporting a low prevalence of DM in SCA patients did not include the abovementioned unreliability of HbA1c tests.

Life expectancy

Reduced life expectancy was initially believed to account partly for the absence, or low prevalence of T2DM, in patients with SCA. This resulted from the observation that most patients suffering from SCA present a shorter life span [39] and would not live long enough to develop T2DM the risk of which increases with age. This assumption is presently being challenged by the emerging reports which show that the life span of SCA patients with SCA have now have improved. This longevity observed in SCA could, in turn, be largely attributed to the treatment advances, such as immunization, stroke prevention, chronic blood transfusion, as well as healthy lifestyle, strong compliance to the treatment regimens, greater family support, stem cell transplantation and gene therapy [40–42].

Insulin resistance (IR) in patients with sickle cell anaemia

Insulin is an anabolic hormone with a number of classic and novel biologic effects. Impairment in all, or some of the effects mentioned above results in IR [43–45]. The complexity of IR is enormous, since it can result from various abnormalities, including defects in insulin receptor and its signal proteins [46].

Different attempts have been made to investigate the relationship between IR and SCD. Alsultan et al. [47] reported that the level of an IR index, i.e. the homeostasis model assessment of insulin resistance (HOMA-IR), was significantly elevated in patients with SCA. In contrast, the findings included in the report are in opposition to the earlier report of ter Maaten et al. [48] which indicated that the insulin sensitivity status in patients with SCA and in the controls was comparable. However, our reports [16, 49] and that of Yavropoulou et al. [50] corroborate the report of ter Maaten et al. [48] demonstrating that patients with SCA have similar insulin sensitivity status as controls, but appear to present a β-cell dysfunction with a reduced insulin secretion. These observations indicate that patients with SCA may not be more predisposed to developing T2DM, despite the associated chronic inflammation. Although reasons for this observation are still poorly understood, compensatory hemodynamic state, which is characterized by vasodilation, could account for the comparable insulin sensitivity status [48].

Body weight

In general, overweight and obesity are not commonly associated with SCA, as it is more linked with stunting and wasting [42]. This is partly due to a high resting metabolic demands, reduced intake of nutrients, which may result from a reduced appetite, recurring illness and hospitalization, as well as chronic inflammation [16, 51, 52]. However, certain reports have shown that overweight and obesity may be present in terms of this disease, and are becoming prevalent among children and adults suffering from SCA [52–55]. This is believed to be related to exercise intolerance, physical incapacity due to sickle cell-related complications, or medical conservatism [53].

The global epidemic of obesity continues to fuel the rising incidence and prevalence of T2DM. Although not all individuals with obesity develop T2DM, the links between excess body weight and T2DM have been well established [56]. The inextricable links involve pro-inflammation, impaired fatty acid metabolism and dysfunction of cellular processes including endoplasmic reticulum stress and mitochondrial dysfunction [57].

These complex factors induce insulin resistance and failure of β-cell consistent with extensive metabolic interplay between the hypothalamus, adipose tissue, pancreas, liver and the skeletal muscles [56, 58–60].

Despite the association between obesity and T2DM, new reports are not yet available indicating that the claimed increase in the incidence and prevalence of excess body weight in SCA individuals facilitates a rise in the incidence and prevalence of T2DM in this group. Therefore, it is important to consider whether the mechanisms of interaction between obesity and T2DM in people with SCA differ from those in people without SCA? This may not be entirely true as SCA is characterized by chronic inflammation which is also a key factor in the pathogenesis of T2DM.

Chronic inflammation, sickle cell anaemia and type 2 diabetes mellitus

Inflammation is a complex physiological response of an organism to harmful stimuli, such as pathogens and damaged/necrotic tissues in order to re-establish homeostasis. It involves the synchronization of activities of many cell types and mediators the response of which depends on the nature of the initial stimulus [61, 62]. Reports have shown that an elevation in inflammatory markers, such as tumour necrosis factor-alpha (TNF-α) and C-reactive protein (CRP), is commonly observed in patients with SCA even in steady state. Thus, chronic inflammation is considered a prominent feature of SCA [15, 51, 63, 64].

The interplay between inflammation and IR/T2DM has been well recognized [65, 66]. Adipocytes and adipose tissue infiltrating macrophages release a number of pro-inflammatory cytokines and chemokines, including as interleukin-6 (IL-6), interleukin-1 beta (IL-1β) and TNF-α. These cytokines exert paracrine effects on insulin target cells to activate inflammatory pathways resulting in the activation of Jun N-terminal kinase (JNK), inhibitor of κB kinase (IKK-β) and other serine kinases. In turn, these kinases phosphorylate insulin receptors, insulin receptors substrate-1 (IRS-1) and other insulin signalling molecules on serine (rather than the normal tyrosine phosphorylation) thereby disrupting the downstream insulin signalling cascades, which consequently results in cellular insulin resistance [67, 68]. Although cytokines usually mediate IR via local paracrine effects, some studies indicate that the tissue cytokines may escape into the circulation and exert endocrine effects by impairing insulin sensitivity in the distal tissues [69].

Inflammation induction in patients with SCA is principally, not adipocentric. Some factors, such as endothelial and coagulation activation, as well as oxidative damage in the cell membrane, which are induced by the SCA-associated intracellular haemoglobin polymerization, have been identified as inflammation inducers [51, 70–72]. Although SCA-associated inflammation is not adipocyte dependent, the possibility of IR induction by cytokines which escaped into the circulation (as found in obesity-inflammation-IR interplay) suggests that SCA-associated inflammatory mediators could also have some IR inducing properties. This issue could even be exacerbated by the emerging reports of overweight and obesity in patients with SCA. Surprisingly, SCA-associated inflammation does not seem to induce IR development as obesity-associated inflammation. This conclusion is supported by reports demonstrating that IR may not be a common

feature in patients with SCA [16, 49, 50]. Moreover, they indicate that there is a need for more research which will further investigate the seemingly metabolic-quiescent nature of inflammation in SCA, which subsequently could become a potential pharmacotherapeutic approach.

Abnormal haemoglobin as a blood glucose buffer

Abnormal haemoglobins may possess an increased blood glucose buffering capacity [73]. It was shown in an *in vitro* study that abnormal haemoglobins can serve as glucose buffer, hence, averting hyperglycemia, as well as its associated complications [74]. This observation is further supported by Al Harbi et al. [73] who demonstrated that patients with sickle cell trait (SCT) seem to be protected against diabetic retinopathy development and progression. Furthermore, they showed that the SCT group presented a reduced prevalence of diabetic macular oedema (DME) and/or proliferative diabetic retinopathy (PDR) compared with individuals with normal haemoglobin. Additionally, they also showed that the absence of SCT and a longer duration of DM independently predicted PDR and/or DME compared to hypertension, nephropathy or diabetes duration.

The possible underlying explanation for this apparent glucose-buffering property of abnormal Hb is that abnormal Hb may exhibit dissimilar biological properties when glucose-bound. Their poor stability when glucose-bound, could activate diverse biological activities which may be protective in terms of the development of hyperglycaemia and its associated complications [74]. Since Hb in SCA is less stable than Hb in SCT, it could thus be inferred that Hb in SCA may have more glucose-buffering property and this may be one of the mechanisms explaining the rare coexistence of SCA and DM. Therefore, once it is properly understood, this novel blood glucose-buffering property could be further explored as a pharmacotherapic approach for DM [73].

microRNAs (miRNAs)

miRNAs are 22-nucleotides containing non-coding RNAs presenting hormone-like activities, as well as regulating the activity of host cells. Most miRNAs are processed into precursor miRNAs and mature miRNAs after the initial transcription of DNA sequences into primary miRNAs [75]. The inhibition of gene expression by miRNAs has been well established. For instance, low molecular weight miRNA-induced silencing complex (miRISC) can induce nuclear degradation of mRNA by interacting with mRNAs within the nucleus [76, 77].

Although data on the mechanisms through which sickled erythrocytes offer protection against DM is scare, it is speculated that miRNAs in the sickled erythrocytes could block mRNA translation of the antibodies which results in the autoimmune destruction of the pancreas [78]. This, in fact, could be a major factor accounting for the rare coexistence of SCA and T1DM.

The DM-protective role of miRNAs in individuals with abnormal haemoglobin could be associated with the protective advantage of SCD against malaria. LaMonte et al. [79] showed that the translocation of sickle cell erythrocyte microRNAs into plasmodium inhibits ribosomal loading, which results in the translational inhibition of parasitic growth proteins resulting in impaired growth of *Plasmodium falciparum*. This role of miRNAs in DM protection should also be further explored as a pharmacotherapy option with regard to DM.

In addition, the beta-globin gene and insulin gene have been mapped to the short arm of human chromosome 11 [80]. However, the possible inhibitory effect between the genetic loci of insulin and beta-globin gene is presently poorly understood, and thus could be further investigated in order to gain more insight into the interplay between SCD and DM [78].

Conclusion

Sickle cell anaemia is associated with several complications and endocrinopathies, although its coexistence with DM continues to be rarely observed. This unexpected particular form of 'protection' against DM represents a clinical puzzle which requires further scientific clarification, whereas its understanding might provide some therapeutic insights for DM.

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REVIEW PAPER

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Allergy to the iodinated contrast media – the clinical and immunological aspects – a literature review

Dorota Jenerowicz
Department and Clinic of Dermatology, Poznan University of Medical Sciences, Poland
https://orcid.org/0000-0002-9833-2454

Krzysztof Kanabaj
Department and Clinic of Dermatology, Poznan University of Medical Sciences, Poland
https://orcid.org/0000-0001-8383-1181
Corresponding author: kkanabaj@gmail.com

Zygmunt Adamski
Department and Clinic of Dermatology, Poznan University of Medical Sciences, Poland
https://orcid.org/0000-0001-6448-0216

Magdalena Czarnecka-Operacz
Department and Clinic of Dermatology, Poznan University of Medical Sciences, Poland
https://orcid.org/0000-0002-4662-2329

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ABSTRACT

Aim. The aim of this paper is to analyse and review the currently available evidence and research with regard to allergy to the iodinated contrast media, which still remains an important, albeit rare, clinical complication.
Material and Methods. We performed our research using the PubMed search engine provided by the National Centre for Biotechnology Information, having inserted 'iodinated contrast media', 'allergy', 'adverse reactions' as the keywords.
Results. Even though the modern iodinated contrast media are much safer than those used in the past, adverse reactions still occur in up to 1–3% of patients undergoing radiological procedures. Their range varies from skin changes, such as a macular rash, prurigo or urticarial, to the more severe multisystemic reactions including anaphylactic shock. The underlying mechanisms are still investigated and are not fully comprehended, although the most frequently accepted explanations include a systemic inflammatory reaction associated with increased histamine and tryptase levels, activation of memory T cells and both direct and indirect damage to the vascular epithelium. The significance of classic allergy tests has not yet been fully established. The associated known risk factors are of various character and researchers have come with different, occasionally contradicting results regarding patients' age and gender, however, other factors have been more clearly described, and include concomitant conditions and medications.
Conclusions. The aforementioned data emphasizes the importance of clinical aspects of allergy to the iodinated contrast media for every practicing physician, as more and more medical specialties benefit from the advantages of modern vascular imaging.

Introduction

The discovery of electromagnetic radiation with the length of 0.01–0.05 nm (Roentgen rays) in 1895 allowed their use in medical diagnostics. X-rays initially proved to be applicable primarily in diagnosing bone diseases, whereas the differences in their absorption by soft tissues were frequently too small to show particular lesions. Therefore, shading agents were introduced in order to properly visualise the pathology of organs in the imaging of the digestive system, circulatory system or urinary tract.

ICMs (iodinated contrast media) are highly concentrated solutions with a low molecular weight. The intravenous contrast media typically contain 270 to 370 mg of iodine/ml, and their doses range from 50 to 150 ml in adults. In fact, it is believed that chemical compounds containing elements with an atomic number of 50–60 are best suited for X-ray diagnostics. The substances which are mostly used nowadays are presented in **Table 1** [1].

Contrast media diffuse rapidly and approximately 70% of the administered dose disappears from plasma within 2–5 minutes after injection. These compounds are eliminated mainly via glomerular filtration (90% of the dose present in urine after 24 hours). Furthermore, the degree of protein binding is small (1–3%), non-specific and applies to water-soluble agents [2–5].

Currently, mostly iodine- and gadolinium based contrast agents are used in modern radiology. Iodine-based media can be divided according to their osmolarity (hiper-, izo- and hipo-), ionicity (ionic and non-ionic), as well as to the number of benzene rings (monomer and dimer). Nevertheless, the safest media are mostly non-ionic and either hypo- or isotonic, as they show significantly less adverse reactions in comparison to their hipertonic counterparts. Nowadays, more than 600 million radiological examinations are currently performed annually worldwide, out of which 40–70 million require the use of various contrast media.

Material and Methods

We performed our research using the PubMed search engine provided by the National Centre for Biotechnology Information, having inserted 'iodinated contrast media', 'allergy', 'adverse reactions' as the keywords.

Epidemiology of hypersensitivity to ICMs

Extensive research conducted in the 1980s allowed to assess the incidence of mild, immediate reactions in 3.8–12.7% of patients receiving high osmolality, ionic ICM injections, as well as in 0.7–3.1% of patients receiving low osmolality nonionic ICMs. As a result, the incidence of serious immediate adverse reactions was estimated at 0.1–0.4% for ionic ICMs and 0.02–0.04% for non-ionic ICMs. The mortality rate is 1 in 100,000 contrast-enhanced examinations, and in spite of the generally higher intensity of response to the ionic agents as compared to the non-ionic ones, it is not statistically significantly different for both ICM groups [2].

The incidence assessment of delayed adverse reactions is slightly more difficult, as indicated by a large discrepancy in the obtained percentages: 0.5–23%. If different, frequently uncharacteristic, adverse reactions occur hours or even days following the diagnostic procedure using ICM, the

Table 1. Osmolality, iodine ratio and iodine content in the iodinated contrast agents [1]

Name	Type	Osmolality [mOsm/kg H_2O]	Iodine ratio	Iodine content [mg/ml]
metrizoate 370 (Isopaque)	ionic monomer	2100	0.5	370
diatrizoate (Renografin)	ionic monomer	1570	0.5	300
iopromide 370 (Ultravist)	non-ionic monomer	774	3.0	370
iohexol 300 (Omnipaque)	non-ionic monomer	672	3.0	300
iomeprol 350 (Iomeron)	non-ionic monomer	618	3.0	350
iohexol 240 (Omnipaque)	non-ionic monomer	518	3.0	240
iodixanol 320 (Visipaque)	non-ionic dimer	290	6.0	320

Iodine ratio: ratio of iodine atoms to particles in solution; Serum osmolality: 285–295 mOsm/kg

actual assessment of a cause-and-effect relationship may pose some difficulty. According to the majority of researchers, such skin lesions in the form of various types of rash clearly suggest the relationship with exposure to ICMs, and their incidence is estimated at 1–3%.

The risk factors for hypersensitivity to ICMs are varied. In fact, *the occurrence of an adverse reaction after the exposure to contrast medium in the past* is considered to be the most vital predisposing factor. Moreover, it is estimated that administering the same or a similar ionic ICM to such a patient involves a risk of reacting again in 21–60% (according to some studies 16–44%) cases, whereas if a person receives a non-ionic agent, the risk of an adverse reaction decreases nearly tenfold. Other risk factors *for an immediate reaction* include:

- **history of allergy**: the most serious risk factor in this group is asthma [2, 6–8];
- **female gender**: an ambiguous and discussed risk factor – according to some reports important for anaphylactoid reactions, according to other reports – for delayed reactions [2, 3, 5–7, 9];
- **race:** British studies indicate a significantly higher risk of ICM hypersensitivity in Indians compared to the inhabitants of northern Europe or Africa [9];
- **age:** literature data are inconclusive – Japanese studies suggest higher incidence of hypersensitivity in young individuals (20–29 years), while according to the British studies, young adults are more likely to experience adverse reactions of mild or moderate severity, whereas older age groups are more likely to suffer severe reactions [6, 9];
- **route of administration of the contrast medium**: risk factors are quite rarely analysed, however, the intravenous route appears to involve a higher risk of adverse reactions in comparison to the intra-arterial administration [10];
- **comorbidities:** the major predisposing factors include cardiovascular diseases (coronary heart disease, rhythm disorders, cardiomyopathies, pulmonary hypertension, hypertension, previous myocardial infarction), as well as mastocytosis, accompanying viral infection on the day of exposure to ICM, and the autoimmune diseases (e.g. systemic lupus erythematosus) [2–9];
- **concomitant medications:** mainly beta-blockers (according to Lang D. et al. the increased severity of anaphylaxis was observed in patients receiving beta-blockers due to an increased propensity to bronchospasm and a decreased cardiac contractility with perpetuation of hypotension and bradycardia) [8].

Factors predisposing to the occurrence of a *delayed* reaction to ICMs mainly comprise: an adverse reaction to ICMs in the past, concomitant recombinant IL-2 treatment (for instance, due to metastatic renal cell carcinoma or melanoma), elevated serum creatinine level (>2 mg/dL) and a positive history of contact allergy [2, 3, 5–7].

Proposed mechanism of hypersensitivity to ICMs

The main mediator involved in the etiopathogenesis of immediate adverse reactions appears to be histamine, which fills the granules of mast cells and basophilic granulocytes. The release of the mediator probably occurs through 2 main mechanisms: explosive degranulation associated with the presence of allergen-specific IgE antibodies, as well as through non-immunity reactions (strictly dependent on the agent dose, to which the cells are exposed). Furthermore, basophils appear to have a greater tendency to release mediators under the influence of non-specific factors than tissue mast cells, whereas in the course of reactions mediated by the elements of the immune system, the simultaneous release of histamine and tryptase occurs. In fact, basophils and mast cells show differences in the content of tryptase in granules (<0.05 pg/cell and 12–35 pg/cell, respectively). Determining plasma histamine concentration shortly after the ICM reaction allows to assess the degree of release of this mediator *in vivo*, while the measurement of serum tryptase concentration, if elevated, suggests the stimulation of mast cells [11].

Early adverse reactions appear mostly 5–15 minutes after the administration, whereas delayed ones – within 3 hours up to 2 days hereafter [2].

According to the Norwegian researchers, T cells are actively involved in the pathomechanism of at least some of the delayed ICM reactions. The clinical picture of this type of reaction is characteristic and includes primarily cases of maculo-

papular skin changes. On the histopathological examination of the skin sample taken from the site where a positive skin test result was obtained with ICM present, there is usually a rich inflammatory infiltration present, consisting of lymphocytes and acid-absorbing granulocytes, as well as features of keratinocyte apoptosis. According to some researchers, the evidence of a significant role of T cells is the fact that adverse reactions to ICMs are more frequent in patients previously treated with IL-2 and in patients with systemic lupus erythematosus. Therefore, it appears that these factors may lower the lymphocyte activation threshold by increasing cytokine production and stimulation of monocytes. Moreover, the obtained positive results of diagnostic tests, such as SPT (skin prick test) and IDT (intradermal testing) with delayed reading, frequently constitute an important evidence of the contrast-specific T cells involvement in the adverse. It is also known that ICMs have the ability to stimulate the proliferation of *in vitro* lymphocytes (obtained from patients with a history of adverse reaction), and T-cell clones ($CD4^+$ and $CD8^+$) specific to a particular causative ICM are obtained from the cultures of these cells. In addition, in contrast to patients presenting symptoms of immediate hypersensitivity to ICMs, patients who have experienced a delayed reaction often present reactivity not only to causative ICM in the subsequent diagnosis, but also to many other contrast agents, which results from the apparent presence of T cells, characterised by a wide panel of cross-reactivity [12–16].

A rare type of a delayed reaction to ICMs is vasculitis. According to some researchers, the essence of this phenomenon may be the induced by ICM precipitation of the circulating immune complexes present in the skin vessels. So far, it has been impossible to obtain positive intradermal tests with ICM, or to confirm the presence of serum IgG and IgM antibodies showing affinity for ICM molecules in patients presenting with signs of vasculitis. Therefore, the abovementioned reaction mechanism requires further clarification [16].

"It has been proven that T lymphocytes are stimulated by increased IL-5 production, which is a growth stimulating factor for acid-absorbing granulocytes. Hence, in some patients eosinophilia can be observed in blood samples, regardless of the coexisting symptoms [17, 18]".

Literature reports dealing with the etiopathogenesis of adverse reactions following contrast media are varied and often contradictory. The controversy is particularly related to the pathomechanism of immediate reactions. It is agreed that these reactions are predominantly associated with the massive release of histamine and other mediators from basophils and mast cells. Nevertheless, the degranulation of these inflammatory cells may be the result of various factors: direct membrane interaction of ICM (especially if the osmolality of the compound is significant), activation of the complement system, or it may be associated with an IgE-dependent reaction [4, 20–24].

Some studies indicate that the majority of adverse reactions to ICM, in particular those of minor severity, may be related to the direct, non-specific effects of ICM molecules. On the other hand, in more severe cases, which occur less frequently, the involvement of an immediate mechanism with IgE antibodies is suspected. However, many authors have long (1950s) denied the inducing effect of ICMs on the production of IgE class antigen-specific antibodies. There are many arguments against it, including the fact that ICMs are not bound by plasma and tissue proteins, and the likelihood of these compounds forming complete protein-hapten conjugates is very low. Moreover, the occurrence of hypersensitivity to ICM at its first application in some patients is also puzzling [6, 17, 25].

In contrast, only a few studies have been able to confirm the presence of serum IgE class antigen-specific antibodies with reference to ICM molecules. In patients with a history of early, severe ICM reaction, some authors observed the coexistence of the elevated circulating histamine and tryptase, positive skin tests, and detectable serum IgE antigen-specific antibodies with respect to the causative contrast medium. This, in turn, with some additional elements of medical history (greater severity of the reaction upon re-exposure and failure of the premedication used) is characteristic of IgE-weighted reaction. According to Gell and Coombs, the mechanism of I hypersensitivity reactions may also be supported in some cases by the correlation of the histamine release rate with the severity of the adverse reaction and a similar rate of recurrence of ICM reactions after previous exposure, as in patients

presenting with symptoms of IgE-dependent allergy to the venom of *Hymenoptera* insects, who were subjected to a provocation test with an allergen (39%). However, it should be emphasised that the percentage of patients who did not show a reaction to ICM at the first administration, but reacted with symptoms of hypersensitivity at the subsequent exposure, is estimated at about 21% [4, 6, 17, 21–23, 26, 27].

The relatively frequent phenomenon of hypersensitivity to ICMs following the first administration in individuals previously not exposed to contrast agents, still remains unexplained. It seems that, in this case, ICMs do not elicit an initial immune reaction, but instead interact with the easily activated memory T lymphocytes, which possess matched receptors. However, the time for an adverse reaction to occur will depend on the individual number of such cells in each patient. Furthermore, some researchers also suggest the possibility of earlier sensitization of the patient to compounds resembling the ICM molecule, for instance halogen derivatives of the benzene ring present in food additives (e.g. erythrosine – a cherry-red food dye), pesticides and herbicides; nevertheless, the initially published observations were not supported by further research [2, 3, 5].

There are a few literature reports relating to the described aspects. In fact, Stellato et al. [28] evaluated *in vitro* the degree of histamine, tryptase and prostaglandin D2 and C4 leukotriene released from human mast cells from the lungs, skin and heart muscle. The authors emphasise the heterogeneity of the reactivity of cells with various origins (for instance, pulmonary mast cells, but not skin mast cells, were degranulated when exposed to ioversol and ioxitalamic acid). Moreover, an important factor favouring the release of mediators from basophilic granulocytes was the hyperosmolarity of ICM, whereas for mast cells such a clear relationship was not found.

Peachell and Morcos [29] observed the in vitro release of histamine from basophils, pulmonary mast cells and cutaneous mast cells from healthy volunteers following various ICM, with a significant positive correlation with the osmolality of ICM, the dose of the agent, and the ICM exposure duration. Thus, it also appears that stronger degranulation of these cells occurs under the influence of ICM with a greater chemical complexity of the molecule.

In a recently published critical analysis of the effects of in vitro coronary angiography, Mansi [30] have emphasised that this is undoubtedly an invasive procedure, as a result of which there is a direct nonphysiological contact of the ICM solution with the vascular endothelium, In addition, foreign bodies in the form of polyurethane or polyethylene catheters also have a direct impact on the endothelium. Transient replacement of blood by ICM may result in a decrease of NO production in response to the mechanical stimuli and vasodilation, but also to an P-selectin expression increase within the endothelium, as well as to an increase in leukocyte adhesion, as well as an increase in the concentration of post-inflammatory cytokines (TNF-α). Intravenous administration of ICM has affects the concentration of various vasoactive substances: there is an increase in the release of kallikrein, bradykinin, serotonin, leukotriene B4 and, particularly importantly, histamine. In addition, an increase in the concentration of C-reactive protein, serum amyloid protein A (serum amyloid protein A – SAA) and IL-6 is observed in patients with unstable angina pectoris following coronary artery imaging without any complications. Therefore, it can be concluded that both mechanical and chemical stimuli acting in the course of coronary artery imaging with the use of ICM elicit a transient inflammatory reaction. According to the author, although millions of coronary angiography procedures are performed annually with a low rate of acute complications, it is also crucial to address the possible distant effects of intense release and interaction of non-inflammatory substances, depending on the initial degree of activation of the immune system. In fact, it is possible that subsequent coronary angiography procedures have a post-inflammatory effect, contributing to the progression of coronary artery atherosclerosis. However, little is known regarding the actual clinical implications of the described phenomenon in relation to the subsequent adverse hypersensitivity reactions to ICM.

It was found that mast cells constitute a crucial element of the ongoing inflammatory process within atherosclerotic plaques, in particular those located in coronary vessels. The mast cell density appears to be directly proportional to the severity of the atherosclerotic process. In fact, mast cell activation may be the result of complex interactions with the associated lymphocytes and mac-

rophages, and may also stem from stress. Furthermore, some authors consider tryptase to be a valuable screening marker for the risk of stable angina pectoris in asymptomatic patients. It may also serve as an exponent of the effectiveness of drugs aimed at stabilising atherosclerotic plaque in patients with the already diagnosed stable angina pectoris.

Clinical signs of hypersensitivity to ICMs

Depending on the responsible pathomechanism, the clinical manifestation of adverse reactions to ICMs is varied (**Table 2**). Immediate hypersensitivity reactions represent the most common (in nearly 70%) cause of urticaria and angioedema, whereas in more severe cases the symptoms affect the respiratory and cardiovascular systems, and also include the anaphylactic shock [2].

As mentioned above, delayed reactions are mostly manifested by maculopapular skin lesions (in more than 50% of cases), and the possible symptoms of erythema, delayed urticaria, sometimes accompanied by scaling. Typically, these reactions are self-limiting and not too severe, although there are also reports in the literature of severe SJS (Stevens-Johnson syndrome), TEN-type (toxic epidermal necrolysis) reactions, or vasculitis caused by ICM. Additionally, biphasic reactions (signs of a delayed reaction with the associated angioedema of the face) have also been reported rarely.

It is also vital to note that other types of adverse reactions to ICM may occur, which are not directly related to the hypersensitivity, such as induced hyperthyroidism [1].

Diagnostic procedures for the suspected hypersensitivity to ICM

In 2005, researchers from the EAACI Interest Group, dealing with medication hypersensitivity (ENDA – European Network on Drug Allergy), developed a valuable set of recommendations for patients diagnosed with hypersensitivity to ICM [2]. It is known that the management will be slightly different in relation to the acute reaction, and other diagnostic elements will have to be taken into account in the case of a delayed reaction. A thorough interview allows for the preliminary assessment of the nature of the reaction based on the time since the intravenous ICM administration to the adverse reactions occurrence (immediate reaction: up to 1 hour, delayed reaction from 1 hour to 7 days). Additionally, the *Ring and Messmer* scale (1997) [24] is effective in the assessment of the clinical condition in the course of an acute reaction. On the other hand, the intensity of a delayed reaction can be assessed on the basis of the simple system proposed by ENDA (**Table 3**).

Further diagnostic procedures in adverse reactions to ICM include:

- during or shortly after the reaction
  - determination of **histamine** and **tryptase concentration levels** – particularly useful in immediate adverse reactions

Table 2. Clinical manifestation of hypersensitivity to ICMs, including immediate and delayed reactions

Immediate reactions	– Pruritus – Urticaria and angioedema – Sudden erythema (flush) – Vomiting, diarrhoea – Rhinitis/congested nose – Voice alteration, cough – Dyspnoea, tachycardia, arrhythmia, hypotension – Shock, cardiopulmonary arrest
Delayed reactions	– Pruritus – Urticaria and angioedema – Skin lesions: maculo-papular, spotty – Stevens-Johnson Syndrome – Lyell's syndrome – Graft versus host reaction – Vasculitis

Table 3. Clinical assessment of a delayed adverse reaction to ICM

Reaction severity	Characteristics
Mild	No treatment required
Moderate	There is a rapid improvement after the started treatment and there is no need for hospitalisation
Severe	The reaction requires the patient to be hospitalised, is life-threatening or is the cause of death

- peripheral blood laboratory analyses of renal and hepatic functions: it is crucial to bear in mind that other organs apart from the skin may become involved in the adverse reaction
- assessment of the peripheral eosinophilia using the Carpentier method [18, 19]
- assessment of lymphocyte activation exponents: CD25, CD69, HLA-DR (flow cytometry) and determination of CD25 serum levels (soluble IL-2 – IL-2sR receptor) involving the immunoenzymatic method, which are mainly used for scientific purposes

› after the period of remission

- skin prick tests with the undiluted contrast medium
- intradermal tests with diluted ICM: 1:10,000 to 1:10 – for the immediate-type reactions
- epidermal patch tests with the undiluted contrast medium – for the delayed-type reaction
- determination of IgE antigen-specific serum concentration: currently no commercial kits for routine measurements are available and the effectiveness of the test still requires assessment in further studies
- assessment of basophil activation: CAST ELISA method showed an increased release of cysteinyl leukotrienes both in *in vitro* and *in vivo* by ICM in patients who showed symptoms of an adverse reaction
- lymphocyte Transformation Test (LTT): requires appropriate laboratory conditions and experience (for a detailed description of the procedure, see the section on the diagnosis); it is not used in the routine clinical practice.
- provocative trials: in the 1970s, a pattern of intravenous, gradual provocation was proposed, consisting of 0.1 ml doses of subsequent ICM dilutions (starting from 1:10,000), administered at a 15-minute interval until a concentration of 1:1 was reached, then applied in the volume of 1 and 5 ml; the trial may be performed before a full dose of ICM is administered to the patient, and is definitely helpful in identifying patients at risk of adverse reactions; however, it is very

Table 4. The proposed treatment for various forms of allergy to ICM

Urticaria			
Mild reaction (scattered or transient pattern)			
No treatment			
May consider:	Diphenhydramine	25–50 mg p.o.	Or other approved antihistamines
	Fexofenadine	180 mg p.o.	
Moderate and severe reactions			
Monitoring vitals	Preserving i.v. access		
May consider:	Diphenhydramine	25–50 mg p.o. / i.v.	Or other approved antihistamines
	Fexofenadine	180 mg p.o.	
Diffuse erythema			
Monitoring vitals	Preserving i.v. access	Oximetry	Oxygen by mask (6–15 l/min)
Hypotension			
	I.v. fluids (0.9% NacL or Lactated Ringer's)	1,000 ml fast	
Consider:	Epinephrine (i.v)	0.1–1.0 mg	
	Epinephrine (i.m.)	0.3 mg	If iv. Access unavailable
Bronchospasm			
Monitoring vitals	Preserving i.v. access	Oximetry	Oxygen by mask (6–15 l/min)
	Beta-agonist inhaler (i.e. salbutamol)	4–10 puffs (100 mcg/dose)	Or 2.5–5.0 mg using nebulizer

time-consuming and therefore rarely useful in the routine clinical practice.

Treatment

The treatment of allergic reactions following the application of contrast media varies depending on the clinical situation. To summarise it more clearly, it has been presented in **Table 4** [10, 26, 31].

Conclusions

1. The currently used non-ionic, low-osmolar ICMs are characterised by high safety, and rarely cause adverse reactions.
2. Multicentre studies are required to assess the pathomechanism of adverse reactions to ICM, as well as to assess the value of diagnostic tests. An adequate number of patients needs to be analysed.
3. In patients presenting with symptoms of an adverse reaction of mild severity, skin tests performed during the remission period are often negative and do not account for an unambiguous inference of the IgE-dependent mechanism of the immediate reaction.
4. When using tryptase as a marker of anaphylactic reaction to ICM, the possibility of increased baseline levels of this mediator in patients with angina pectoris undergoing coronary angiography and PCI should be considered.

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REVIEW PAPER

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Fluorescent spectroscopy of collagen as a diagnostic tool in medicine

Łukasz Saletnik

Department of Vascular and Internal Diseases, Faculty of Health Sciences, Ludwik Rydygier Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Torun, Bydgoszcz, Poland

https://orcid.org/0000-0001-7847-4351

Corresponding author: l.saletnik@cm.umk.pl

Roland Wesołowski

Department of Medical Biology and Biochemistry, Faculty of Medicine, Ludwik Rydygier Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Torun, Bydgoszcz, Poland

https://orcid.org/0000-0003-2673-8313

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ABSTRACT

Medicine continuously needs to improve the existing diagnostic solutions or introduce new ones. Despite the fact that collagen is a well described protein, collagen-related diseases represent the disorders requiring improvement particularly in terms of research tools. These diseases include connective tissue diseases, keratoconus, where a change in the structure of collagen is observed causing a deformation of the cornea, as well as neoplasms, in which the amount of collagen is multiplied in cells.

Fluorescence spectroscopy constitutes a highly sensitive, non-invasive research method, thus, its use in medicine can contribute to the development of excellent diagnostic methods. This method allows to determine the changing amount of the tested fluorophores, as well as the change of the pH of the environment in which these fluorophores are located. Until now, numerous studies on collagen have been performed using fluorescence spectroscopy. However, a detailed analysis of the literature revealed some discrepancies which have been summarized in this paper.

The collected experimental results allowed to conclude that the discrepancies in the obtained fluorescence excitation and emission spectra of collagen may result from the structural richness of collagen. Another reason for the variability of the results is the different experimental conditions, i.e. the excitation and detection of collagen fluorescence at different wavelengths. Therefore, it should be emphasized that collagen spectroscopy constitutes an extremely promising method, although the determination of the exact conditions of the experiment and their standardization are required in the research on the diagnostic use of this technique.

Introduction

Collagen is the most abundant protein in the human body. Thus, a detailed description of all its properties, including the spectral characteristics, is necessary. This need results undeniably from the fact that many connective tissue diseases are associated with the changes in the functioning and structure of collagen. Other diseases can also be caused at least partly by the functional changes in collagen. An example of this type of disease is an eye condition referred to as keratoconus, where a reduction in the amount of collagen causes the cornea to reshape. Therefore,

a detailed understanding of the spectroscopic properties of collagen may contribute to the development of new diagnostic methods.

Currently, there are no studies with regard to connective tissue diseases using fluorescence spectroscopy. Nevertheless, connective tissue diseases are often associated with collagen impairment. Moreover, changes in tissue collagen fluorescence during the neoplastic process have been demonstrated in many studies. In fact, the experimental results of some experiments indicate that low collagen and high NAD(P)H intensity of fluorescence characterizes high-grade dysplastic lesions, compared to non-dysplastic tissues [1]. These experiments indicate that once the method is standardized, fluorescence spectroscopy can become an indispensable tool in the diagnosis and/or treatment of certain neoplasms as measurements are performed quickly and can be performed *in vivo* in some cases. Therefore, the idea of using fluorescence spectroscopy in the diagnosis of connective tissue diseases seems to be justified and promising.

Over the last few years, a number of spectroscopic studies of collagen have been carried out. However, the issue of collagen spectroscopy has not been sufficiently described in the literature, and reports are inconsistent. Therefore, the aim of this study is to compile and verify the current knowledge related to the stationary fluorescence spectroscopy of collagen.

Literature Search

All available literature databases were searched. The literature was reviewed at PubMed, Medline (Webhost EBSCO) and Web of Science from February 2021 to June 2021. The initial database searches were made against the following keywords: collagen and fluorescence. Other keywords were added subsequently, allowing to specify the research method being sought: spectroscopy, excitation and emission spectra. The analysis was limited to the English language. Papers which do not provide information on collagen excitation and emission spectra were excluded.

As shown in **Figure 1**, the review uses information from 36 papers, 9 of which contain the complete spectra of excitation and / or emission of collagen fluorescence, whereas 20 papers provide additional information on the fluorescent properties of collagen. The remaining 8 articles contain information on the structure of collagen and fluorescence spectroscopy.

Steady State Fluorescence Spectroscopy

The use of fluorescence in life sciences has gained importance over the past thirty years. Fluorescence spectroscopy is regarded as a significant research tool in biotechnology and biophysics [2], with fluorescence detection as an extremely sensitive and specific method. It uses molecules called fluorophores/fluorochromes. Excitation of fluorophores by a light beam of a lower wavelength elevates the electrons to an excited state, which then emit energy as light of a longer wavelength during their return to the ground state [3]. Autofluorescence uses the neutrally occurring chromophores in the tested cells/tissues. Fluorescence spectroscopy allows for the observation of a minimal change: the amount of fluorophore, the environment of the fluorophore and even the conformation of proteins. Fluorescence measurement devices include fluorimeters and spectrofluorimeters. Depending on the method of analysis, measurements can be divided into stationary and time-resolved measurements [2, 3].

The method of fluorescence analysis can be a very useful alternative in the diagnosis of diseases accompanied by changes in the structure, environment or the number of endogenous fluorophores. Most frequently, individual fluorophores differ from each other in the wavelength at which they give the maximum excitation and/or maximum emission of fluorescence [4]. The excitation maximum of the fluorescence is the wavelength at which the absorption of light is the highest, whereas the emission maximum of the fluorescence is the wavelength at which the highest intensity fluorescence is observed. The difference between the position of the maximum of the absorption band in relation to the emission band is known as the Stokes shift [2, 4]. The abovementioned differences often allow for the identification of specific fluorophores. Nonetheless, in the case of stationary measurements, the spectra of

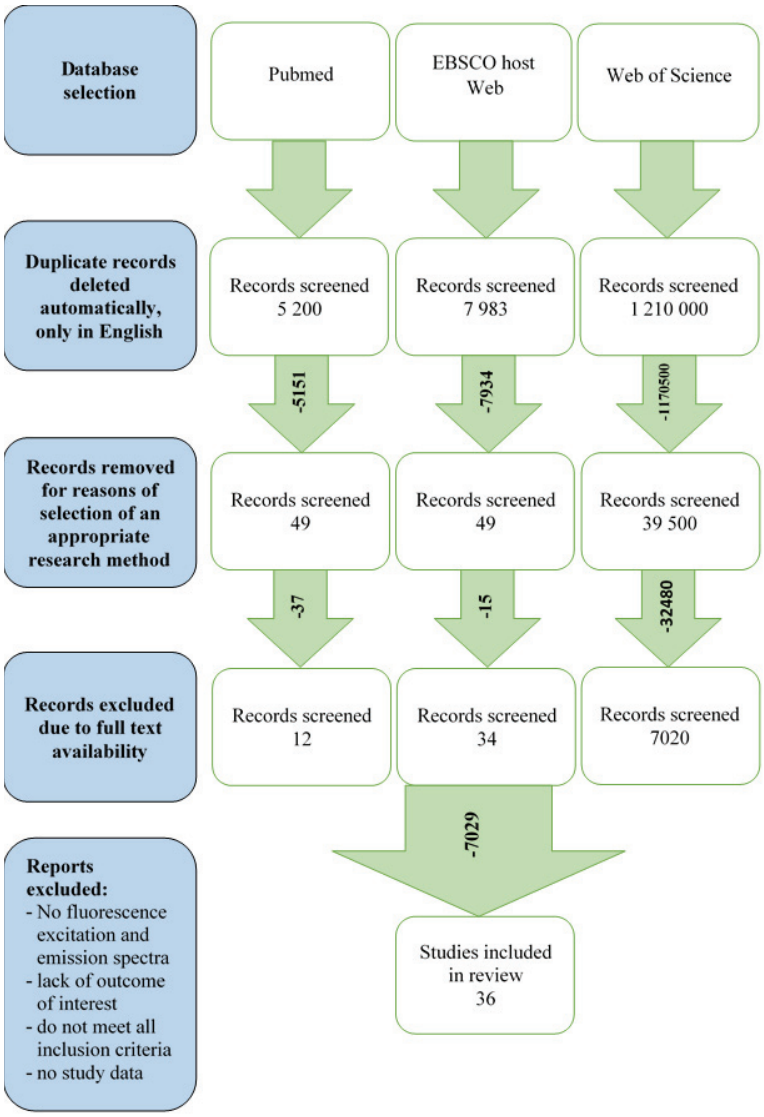

Figure 1. Literature search strategy. As indicated by the search algorithm, information from 36 papers was used

some chromophores may coincide. Therefore, in some cases, much more sensitive measurements of time-resolved spectroscopy may provide a useful extension to the research [3]. However, samples which possess the same autofluorophores in their structure can be distinguished by the amount of chromophores or by the influence of the environment in which these chromophores are located, e.g. a change in the hydrophobicity of the environment may change the spectral properties of the tested molecules. These factors may occur in the course of formation of pathological tissue lesions, as in the case of a neoplasm [4, 5]. Well-known endogenous fluorophores capable of emitting autofluorescence include aromatic amino acids (phenylalanine, tyrosine and tryptophan), molecules of cellular energy metabolism: nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FAD), vitamins, lipids, elastin and collagen, the main component of the extracellular matrix and the most commonly found protein in the human body [2, 5].

An indisputable advantage of the optical methods is their minimal invasiveness, as measurements can be performed *in vivo*. Therefore, there is no cell procurement, or possibly only a small piece of the tissue is removed during surgery. Additionally, blood or urine samples can be also used as research material, whereas real-time research allows to obtain the results immediately. Thus, by using spectrophotometry, the experiment can be performed in a relatively short time.

Collagen is used as a marker of pathological changes in *in vivo* experiments [1, 4, 6, 7]. Due to their availability, the most common experiments involve epithelial tissues, such as the skin [1, 6, 7]. However, with ever greater miniaturization of equipment and more sophisticated research tools, it is possible to examine previously inaccessible locations, such as the colon [4]. The examination involves (1) the insertion of the fiber optic probe as an endoscope, (2) the excitation of the tissue fragment with the light of a certain wavelength / wavelengths, (3) collecting the fluorescence from the tissue into the detector and (4) its further analysis [4]. Hence, as the research on epithelial cells shows, fluorescence spectroscopy is a very promising method of examining pathological changes such as cancer [1, 6, 7]. However, to date it has been limited to locations where fiber optic courts can be introduced. Nevertheless, the increasing miniaturization allows the device for reaching the previously inaccessible sites in the human body.

Collagen structure

Although the understanding of the pathogenesis of many collagen-related diseases remains insufficient, the structure of collagen has been well described. So far, 28 types of collagen have been identified. Some amino acid residues found in the structure of collagen are capable of autofluorescence, and this type of amino acids possesses an aromatic ring in their structure, namely tryptophan, tyrosine and phenylalanine. Furthermore, proline-hydroxyproline-glycine is the most common amino acid sequence observed in the collagen chain. This triplet constitutes about 10.5% of the collagen sequence. Hydrogen bonds formed between proline and glycine residues of adjacent chains link them with each other. The hydrogen bonds help hold three helices together, thus creating a triplet superhelix. Procolagen fibers formed in this way undergo further post-translational processing, the product of which is tropocollagen. Divalent cross-links are formed between tropocollagen fibers with the use of the enzyme lysyl oxidase, and the cross-links may be further modified [8].

In addition to aromatic amino acid residues, the cross-links are also capable of autofluorescence in the collagen structure (**Figure 2**). Since aromatic amino acids can be found in various proteins, the cross-links present in this protein comprise the structural elements determining the importance of spectroscopic studies on collagen [8]. As the human body ages, immature divalent cross-links are converted into mature trivalent cross-links [9]. Moreover, the divalent cross-link may undergo modifications, e.g. glycosylation. Depending on the period of development and external factors to which the examined tissue has been exposed, various modified cross-links are formed. They may be detected due to the differences in the fluorescence spectra of the sample [9–11].

Collagen in its natural form occurs as a solid molecule, insoluble in water and very resistant

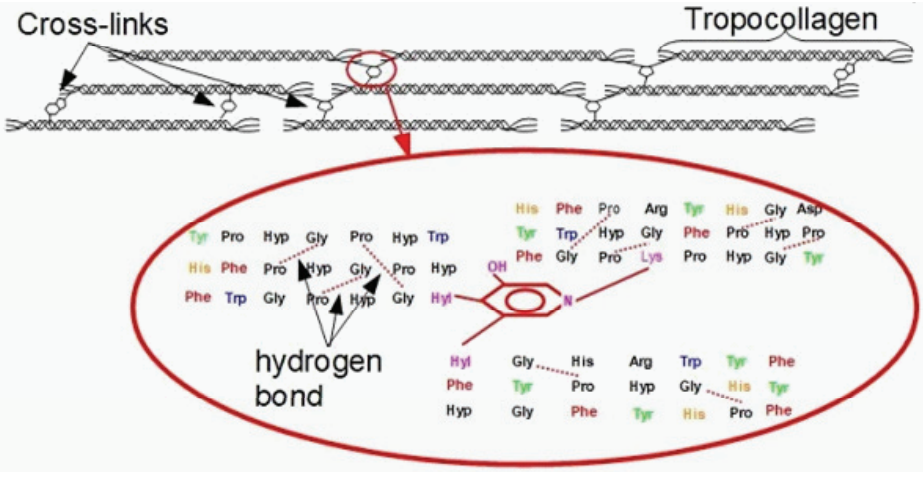

Figure 2. Structure of collagen in which hydrogen bonds (dotted lines) and cross-links between tropocollagen fibrils (center of the Figure) can be distinguished

to degradation [8, 12]. Due to its resistance during isolation, collagen can be divided into acetic acid-soluble and insoluble collagen, the latter of which can be isolated with the use of enzymes, such as pepsin or collagenases [6, 13].

Fluorescence Spectroscopy of Collagen

In the literature, varied information on spectroscopy of collagen can be found. According to the majority of literature data, the absorption maximum of collagen ranges between 340–370 nm [11, 13–15]. For the collagen emission maximum, more diverse data are observed, which indicate that it is 380 nm [14], around 400–410 nm [1], or around 440–450 nm [1, 6, 7, 11, 13]. In various studies, only one peak in the fluorescence emission spectra of collagen has been indicated [1, 6, 7, 11, 13]. However, several other studies have found collagen spectra more complex with several fluorescence peaks [7, 16].

Robert Gillies et al. [6] divided the emission spectra of collagen according to the enzyme used for isolation. Pepsin-soluble collagen was determined to have the excitation maximum at 335 nm and the emission maximum at about 390 nm. On the other hand, collagenase-soluble collagen had the excitation maximum of 370 nm and the emission maximum of 440–460 nm. Smirnova et al. [7] determined the fluorescence spectrum of type IV collagen suspended in water with the emission maximum at 440 nm, and several local peaks at 380, 420, 465 and 495 nm following excitation with the light of approximately 370 nm. Julian Ionita et al. [16] obtained emission spectra of pure hydrolyzed collagen powder with maxima at wavelengths of 430 nm and 460 nm with the local maximum at about 390 nm. Calfskin acid-soluble collagen isolated by Deyl et al. [15] showed the fluorescence maximum at 440 nm and 495 nm and an additional peak at 410 nm, as well as excitation spectra with a maximum at 345, 370 and 410 nm. Researchers from the Massachusetts Institute of Technology determined four excitation/emission wavelength pairs of powder Bovin collagen: (280 / 310 nm), (265 / 385 nm), (330 / 390 nm), and (450 / 530 nm), using a contour map representation of the fluorescence (EEMs) [17]. The summary of these data is presented in **Table 1**.

In order to obtain a consistent representation of collagen fluorescence spectrum data, several publications with excitation / emission spectra of collagen fluorescence were selected [for a complete list of publications see **Figures 3–4**]. Subsequently, on the basis of the figures found in these publications, the excitation as well as emission bands were digitized and compiled together in the collective figures of collagen excitation spectra (**Figure 3**) and collagen emission spectra (**Figure 4**).

Based on the literature data, the absorption/fluorescence spectrum of collagen can be divided into two fractions. The former fraction includes the amino acid residues present in collagen, while the latter belongs to the cross-links. The amino acid fraction can be excited in the range between 250–290 nm, and fluorescence emission spectra can be related to the range from 250 nm to 440 nm [18]. Furthermore, the aromatic amino acids responsible for collagen fluorescence in this wavelength range are phenylalanine [Phe], tyrosine [Tyr] and tryptophan [Trp] [19]. Their ratio of the fluorescence excitation maximum to the fluorescence emission maximum is 258/282 nm, 275/303 nm, and 275/348 nm, respectively. Based on this information, it can be concluded that the fluorescence excitation bands of collagen (marked as No. 2 and 5 line in **Figure 3**) and the fluorescence emission bands of collagen (marked as 4 and 6 line in **Figure 4**) are related to these amino acids [18]. The cross-links form the latter fraction responsible for collagen absorption / fluorescence which includes trivalent cross-links and advanced glycation end-

Table 1. Summary of literature data with regard to the excitation maximum and emission maximum of collagen fluorescence

Reference	Excitation maximum	Emission maximum
[15]	345 nm, 370 nm, 410 nm	440 nm, 495 m
[7]	370 nm	380 nm, 420 nm, 440 nm, 465 nm i 495 nm
[16]	360 nm	430 nm, 460 nm
[17]	280 nm, 265 nm, 330 nm, 450 nm	310 nm, 385 nm, 390 nm, 530 nm

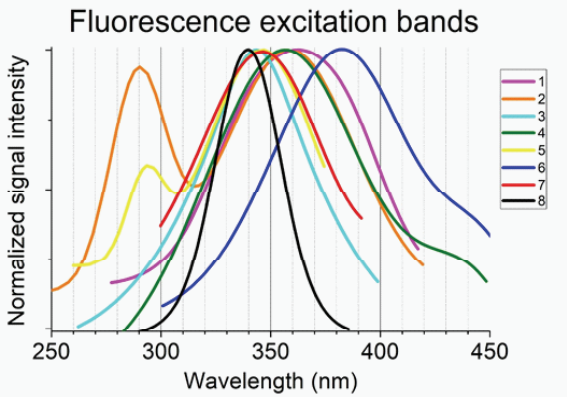

Figure 3. The differences in the excitation bands of collagen prepared by various methods. For this purpose, fluorescence spectra presented in different publications were digitized point by point in order to obtain minimal differences compared to the original bands

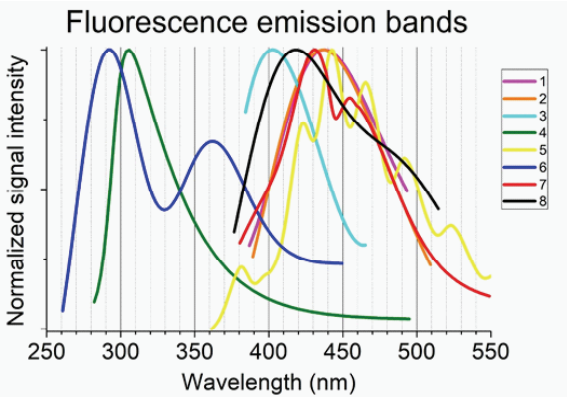

Figure 4. The differences in the emission bands of collagen prepared by various methods. For this purpose, fluorescence spectra presented in different publications were digitized point by point in order to obtain minimal differences compared to the original bands

products (AGEs), varying in terms of their spectrophotometric properties [9].

Collagen cross-links spectroscopy

Until now, several types of cross-links have been identified using HPLC [9]. Nonreducible cross-links, such as pyridinoline (Pyr) or deoxypyridinoline (Dpyr) have been identified using their natural ability of autofluorescence. They are characterized by the maximum wavelength in excitation spectrum at 295 nm, 325 nm and 340 nm (depending on the pH of the environment) with the light emission maximum of 395 nm [20]. Moreover, pentosidine (Pen) is also naturally capable of fluorescence and its excitation emission maximum amounts to 335/385 nm [9, 20, 21]. Another cartilage-specific cross-link, 2,6-dimethyldifuro-8-pyrone (DDP) has been identified and isolated. It is capable of autofluorescence with a maximum ratio of 306/395 nm (excitation/emission) [22]. Additionally, other divalent reducible cross-links, including dihydroxylysinonorleucine (DHLNL), hydroxylysinonorleucine (HLNL), lysinonorleucine (LNL) and trivalent nonreducible cross-link histidinohydroxylysinonorleucine (HHL), have been detected by generating O-phthalaldehyde derivatization because they do not have the ability to naturally fluorescent [9].

According to the literature data, AGEs can constitute the main fraction of cross-links associated with collagen fluorescence [10, 23, 24]. They are responsible for a fluorescent excitation-emission maximum at 360/460 nm [10, 23, 24]. The initial amount of this type of bonds is small, but it increases with age, resulting in the collagen molecule aging. However, in the case of diabetic patients, AGEs are multiplied [9, 25–29]. Pen, as mentioned above, belongs to the well-described AGEs. This cross-link has a ratio of excitation-emission maximum which is significantly different from other AGEs [9, 21]. Other collagen cross-link AGEs capable of autofluorescence include vesperlysine A and B, vesperlysine C, lysyl-pyrropyridine, FFI, Argpyrimidine, Crossline, Fluorolin, and threosidine [21, 27, 29–31]. The wavelengths at which the excitation-emission maxima were measured are summarized in **Table 2**. Among AGEs identified in collagen, cross-links which do not possess a benzene ring in their structure have been described, thus, these cross-links are incapable of autofluorescence, e.g. N- (carboxyethyl) lysine (CEL), N- (carboxymethyl) lysine (CML), methylglyoxal-lysine dimer (MOLD) and glyoxal-lysine dimer (GOLD), and formyl threosyl pyrrole (FTP) [10, 28, 29, 32].

During the development of the organism, divalent immature cross-links are converted into non-reducible mature or glycation cross-links. Due to the very long half-life of collagen in various tissues, e.g. cartilage or skin, mature cross-links and AGEs accumulate with age [9, 27–29, 33]. As reported in the literature, numerous additional AGEs are formed during the incubation of cartilage with threose [10]. Furthermore, AGEs

Table 2. Summary of the identified cross-links capable of fluorescence.

Name of cross-link	Excitation /Emission maximum [nm]	References
Trivalent cross-links		
Pyridinoline (Pyr), Deoxypyridinoline (Dpyr)	295 nm (acid), 325 nm (neutral), 340 nm (alkaline)/395 nm	[20]
Hydroxylysyl pyridinoline (HP), Lysyl pyridinoline (LP)	295 nm / 395 nm	[32]
2,6-dimethyldifuro-8-pyrone (DDP)	306/395 nm	[15,32]
Advanced glycation endproducts (AGEs)		
Pentosidine (Pen)	335 nm/385 nm	[9,21,32]
Vesperlysine A i B	366 nm/ 442 nm	[21]
Vesperlysine C	345 nm/ 405 nm	[21]
Lysyl-pyrropyridine	370 nm/ 448 nm	[21]
FFI	380 nm/ 440 nm	[21]
Argpyrimidine	335 nm/400 nm	[21]
Crossline	379 nm/ 463 nm	[21]
Fluorolink	380 nm/ 460 nm	[21]
threosidine	328 nm/ 402 nm	[31]

contribute to the aging of tissues (cartilage, skin or bones) and changes in their properties, e.g. their ability to deform [10, 28, 29]. Additionally, the results of various experiments indicate that cross-links, such as CML, CEL and Pen, constitute only about 4.7% of the newly formed AGEs [10].

Conclusion

The discrepancy in the literature data on collagen spectroscopy does not entirely imply that they are incorrect. In fact, different results of spectrophotometric tests of collagen may stem from collagen structural diversity and different spectrophotometric parameters of the experiment. Therefore, the result of the experiment is closely related to the type of collagen tested, or the physical parameters of the experiment. Thus, it seems essential to define the exact test parameters and identify the collagen type when presenting the experiment, or to provide an accurate description of the investigated tissue, since this may help other researchers relate to the experiment. Moreover, the unification of parameters with regard to the spectrophotometric tests may contribute to a faster progress in research on collagen.

Acknowledgements

List of abbreviations
NADH - nicotinamide adenine dinucleotide
FAD - flavin adenine dinucleotide
AGEs - advanced glycation end-products
Pyr - pyridinoline
Dpyr - deoxypyridinoline
Pen - pentosidine
DDP - 2,6-dimethyldifuro-8-pyrone
DHLNL - dihydroxylysinonorleucine
HLNL - hydroxylysinonorleucine
LNL - lysinonorleucine
HHL - histidinohydroxylysinonorleucine
CEL - N-(carboxyethyl) lysine
CML - N-(carboxymethyl) lysine
MOLD - methylglyoxal-lysine dimer
GOLD - glyoxal-lysine dimer
FTP - formyl threosyl pyrrole
HP - Hydroxylysyl pyridinoline
LP - Lysyl pyridinoline

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

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