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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*.

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ORIGINAL PAPER

JMS Journal of Medical Science

Role of *ARHGAP29* nucleotide variants in the etiology of non-syndromic cleft lip with or without cleft palate

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ABSTRACT

Aim. Non-syndromic cleft lip with or without cleft palate (nsCL/P) is a common birth defect of complex and heterogeneous aetiology. Genome-wide association studies (GWAS) of nsCL/P have identified an association for the 1p22.1 chromosomal region, in which *ARHGAP29* was suggested as a candidate gene. Thus, the current study aimed to determine the contribution of the common and rare *ARHGAP29* nucleotide variants to the risk of nsCL/P in the Polish population.

Material and Methods. In total, 197 common nucleotide variants (SNVs) and 22 missense variants located within the *ARHGAP29* locus at chromosome 1p22.1 were genotyped by SNV microarray. The study was conducted in 269 individuals with nsCL/P and 569 healthy individuals.

Results. Statistical analysis revealed that 31 common nucleotide variants located at the ARHGAP29 locus were significantly associated with the increased risk of nsCL/P. The strongest individual SNV was rs2391467 with a p-value = 2.49E-06 (OR = 1.64, 95%CI: 1.34 – 2.02). Besides, one potentially deleterious missense variant (rs140877322, p. Arg348Leu) was identified in a single patient with nsCLP.

Conclusion. These findings confirm *ARHGAP29* as a strong candidate gene for nsCL/P, with both common and rare nucleotide variants of this gene involved in the aetiology of nsCL/P in the Polish population.

Introduction

Non-syndromic cleft lip with or without cleft palate (nsCL/P) is one of the most common congenital defects in humans, affecting approximately 1/700 live-born children worldwide [1]. The prevalence of nsCLP varies by geographic location, ethnic/racial background, and socioeconomic

status [2]. The complex aetiology of this congenital anomaly reflects the action of multiple genetic factors and environmental exposures, hence it has not been fully elucidated. NsCL/P can be divided into the cleft of the palate only (CPO), and those affecting the lip with or without the palate (CL/P) [3]. Over 500 human syndromes in which clefting is a common feature have been identified (https://www.ncbi.nlm.nih.gov/OMIM/), although most cases correspond to isolated non-syndromic clefts with the absence of other structural or cognitive abnormalities [2]. Previous studies revealed that nsCL/P might have unique aetiological features, including specific genetic associations [2,4], however, the genetic components of nsCL/P have remained elusive due to the influence of multiple environmental risk factors [5].

To date, a variety of research methods have been applied to identify genetic factors contributing to nsCL/P. Genome-wide association studies (GWAS) have been crucial in identifying 40 novel risk loci that show strong associations of single nucleotide variants (SNVs) with nsCL/P [6–10]. The most consistent results in multiple populations were observed for nucleotide variants in *IRF6* (OMIM* 607199) gene, encoding a transcription factor critically involved in craniofacial development [2] and the gene-poor region of chromosome 8q24.21 [11–14]. Also, the chromosomal region consistently associated with nsCL/P is 1p22.1, initially implicating *ABCB4* (OMIM:*601691) as a candidate gene at this locus. However, *ABCB4* was excluded due to its retinal expression and known role in a spectrum of retinal disorders [15,16]. Therefore, it has been hypothesised that a neighbouring gene, *ARHGAP29* (OMIM:*610496), maybe the aetiologic gene within the same region. During craniofacial development in murine embryos, *ARHGAP29* expression was detected in the frontonasal, lateral prominences and palatal shelves [17]. Moreover, there are reports that *IRF6* regulates keratinocyte migration through *ARHGAP29*. Cells lacking the *IRF6* gene had lower levels of *ARHGAP29* and hyperactive Rho GTPase activating protein (GAP), which is involved in crucial cellular functions essential for craniofacial development [12,18]. In addition, sequencing of *ARHGAP29* in patients with nsCL/P identified eight potentially deleterious and aetiological variants, including a frameshift and a nonsense variant [17]. Further functional studies identified a novel missense variant in *ARHGAP29* (c.1654T>C, p.Ser552Pro), showing ARHGAP29 to be a regulatory protein affecting the development of the face [19]. These findings suggest that *ARHGAP29* may play a role as the aetiologic gene at the 1p22.1 locus for nsCL/P [17]. Therefore, this study investigated whether common SNVs and rare missense variants at the *ARHGAP29* locus contribute to the risk of nsCL/P in the Polish population.

Material and Methods

The present study was designed similar to our previous cleft association studies and conducted on the same study population [9,20].

Study population

Patients with a diagnosis of nsCL/P (58.0% of males) were recruited from several Polish medical centres. Among patients, 229 (85.1%) individuals had non-syndromic cleft lip and palate (nsCLP) and 40 (14.9%) individuals had non-syndromic cleft lip only (nsCLO). The control group was composed of 569 healthy individuals (49.6% males) without any developmental anomalies. Detailed characteristics of patients and controls enrolled in the study are presented in **Table 1**. All study participants were of Polish origin. DNA was isolated from peripheral blood lymphocytes by salt extraction. All experimental protocols were approved by the Institutional Review Board of Poznan University of Medical Sciences, Poland [21]. Written and oral consent was obtained from all participants or their legal guardians.

Table 1. Characteristics of study patients and controls

	nsCL/P patients (n = 269)[a]	Controls (n = 569)[a]
Cleft type		
nsCLP	229 (85.1%)	
nsCLO	40 (14.9%)	
Gender		
male	156 (58.0%)	282 (49.6%)
female	113 (42.0%)	287 (50.4%)

[a] Final number of samples analysed in the present study after exclusion of individuals based on stringent quality control criteria
nsCL/P - non-syndromic cleft lip with or without cleft palate
nsCLP - non-syndromic cleft lip and palate
nsCLO - non-syndromic cleft lip only

Common SNV selection and genotyping

Common single nucleotide polymorphisms (SNVs) located within the *ARHGAP29* locus at chromosome 1p22.1 were genotyped with using the HumanOmniExpressExome-8 v1 array (Illumina, San Diego, CA, USA) according to the manufacturer's instructions. After applying stringent quality control criteria (SNV call rate >0.95, minor allele frequency, MAF, >0.05, Hardy-Weinberg, HW, equilibrium p-value >0.001 in controls), 197 common SNVs were subjected to statistical analysis.

Statistical analysis

The association of *ARHGAP29* locus SNVs with nsCL/P was tested with the Cochran-Armitage trend test. Odds ratios (ORs) and corresponding 95% confidence intervals (95% CIs) were used to assess the strength of the association. ORs were calculated for the allelic model (a vs A; a is the risk allele). The Bonferroni correction was applied to account for multiple comparisons, and p-values < 2.54E-4 (0.05 / 197 SNVs) were considered as statistically significant. The pair-wise linkage disequilibrium (LD) between the top was evaluated using the Haploview 4.2 software (www.broadinstitute.org/haploview/haploview, **Table 2**). Separate statistical analyses were conducted for individuals with nsCLP and nsCLO to assess the subgroup-specific effects of the significantly associated SNVs. In addition, separate analyses were performed in male and female groups. The effects of genotype x gender interactions were evaluated by logistic regression approach.

In silico analysis of missense variants

The SNV microarray used in the present study allowed for the genotyping of 22 *ARHGAP29* missense variants. The putative functional consequences of these missense SNVs were analysed *in silico* using SIFT (http://sift.jcvi.org/), PolyPhen (http://genetics.bwh.harvard.edu/pph2/), and Mutation Assessor (http://mutationassessor.org/r3/) tools. Additionally, for all these variants, the frequency of the minor allele was checked in the Exome Aggregation Consortium (ExAC) database (http://exac.broadinstitute.org/).

Results

Common SNVs

Statistical analysis of the 197 ARHGAP29 locus SNVs revealed that 31 were nominally associated (p_{trend} < 0.05) with the risk of nsCL/P (**Figure 1**, **Supplementary Table 1**). Three SNVs, rs11165101, rs11165110 and rs2391467, were statistically significant even after applying the strict Bonferroni correction for multiple comparisons (p_{trend} = 1.71E-04, p_{trend} = 2.19E-04 and p_{trend} = 2.56E-06, respectively). These variants located within the same recombination region were in moderate LD with each other (the mean r^2 = 0.76 and D' = 0.99; **Table 2**). Two other SNVs, rs3789688 and rs2065971, were close to the study significance level (p_{trend} = 8.34E-04 and p_{trend} = 2.91E-04, respectively). The minor allele of the strongest individual SNV (rs2391467) located 14.7 kb upstream of *ARHGAP29* was associated with a 1.64-fold increased risk of nsCL/P (95%CI: 1.34 – 2.02, p = 2.49E-06), with the allelic ORs for the other four top variants in the range of 1.43 to 1.52. For all of them, the major allele was the risk allele. Association results for tested variants are presented in **Table 3** and **Supplementary Table 1**.

Table 2. Linkage disequilibrium values D' and r^2 for the most significant SNVs located at the *ARHGAP* locus

	rs3789688	rs11165101	rs11165110	rs2065971	rs2391467
rs3789688	-	0.78	0.78	0.71	0.83
rs11165101	0.46		1.00	0.98	0.98
rs11165110	0.46	1.00	-	0.98	0.98
rs2065971	0.41	0.89	0.89		1.00
rs2391467	0.61	0.64	0.64	0.71	-

Numbers denote D' and r^2 values expressed as a percentage of maximal value (1.0). D' values are presented above diagonal.
A red-to-white gradient shows highest (1.0) to lowest (0.0) D'. r^2 vales are presented below diagonal.
A black-to-white gradient shows highest (1.0) to lowest (0.0) r^2.

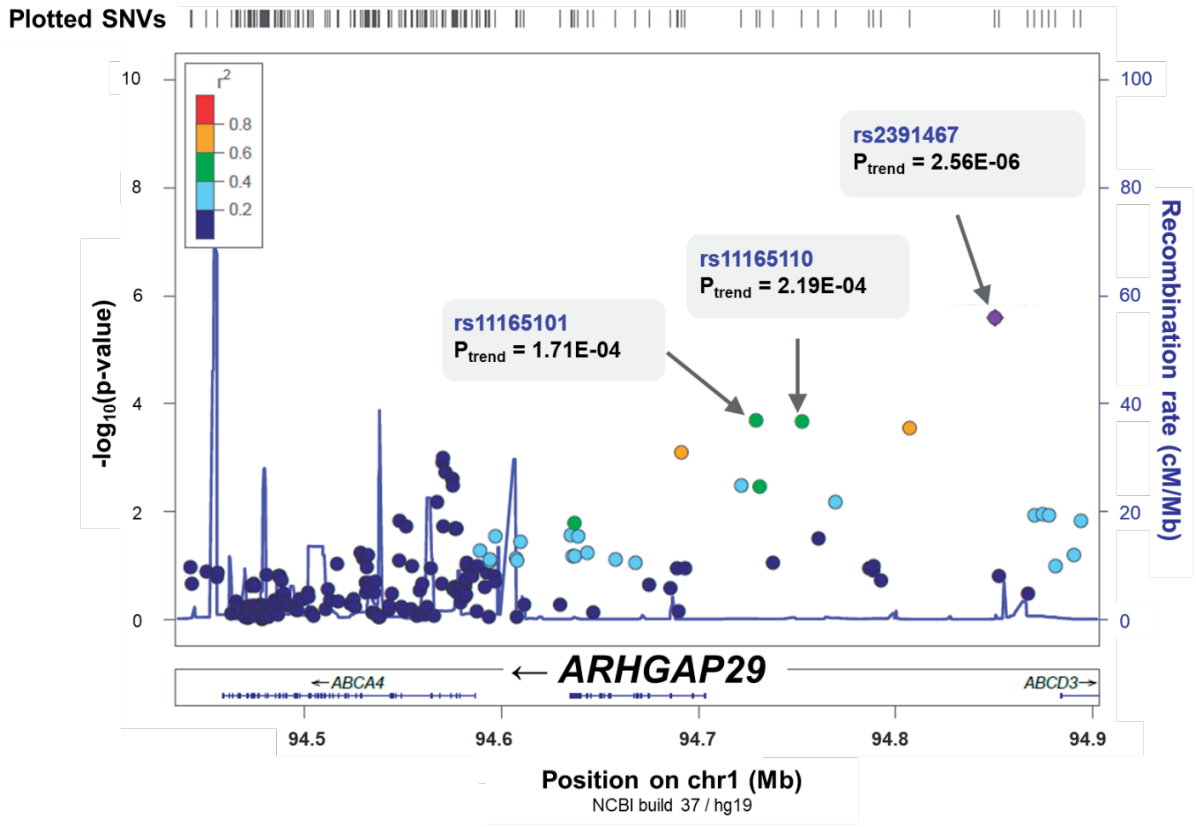

Figure 1. Regional plot of association results within the *ARHGAP29* locus

Table 3. Association results for the most significant variants at the *ARHGAP29* locus (p_{trend} values < 1.00E-03)

rs number	Location (bp)[a]	Consequence type	Gene	Alleles[b]	p_{trend}	RAF nsCL/P	RAF Controls	OR (95%CI)[d]
rs3789688	chr1: 94691240	intronic	*ARHGAP29*	T / C	8.34E-04	0.59	0.50	1.43 (1.17–1.77)
rs11165101	chr1: 94729088	intergenic	*ARHGAP29 / ABCD3*	A / C	**1.71E-04**	0.67	0.57	1.52 (1.23–1.89)
rs11165110	chr1: 94752469	intergenic	*ARHGAP29 / ABCD3*	A / G	**2.19E-04**	0.67	0.57	1.51 (1.22–1.87)
rs2065971	chr1: 94807102	intergenic	*ARHGAP29 / ABCD3*	A / C	2.91E-04	0.65	0.55	1.48 (1.20–1.83)
rs2391467	chr1: 94850443	intergenic	*ARHGAP29 / ABCD3*	A / G	**2.56E-06**	0.58	0.46	1.64 (1.34–2.02)

[a] NCBI build 37 / hg19.
[b] Underline denotes the risk allele (for all variants, exept rs2391467, the major allele is a risk allele).
[c] The ptrend values below 2.54E-04 (0.05 / 197 SNVs) were interpreted as statistically significant.
[d] Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated for the allelic model (a vs A; a is the risk allele).
Significant p-values are highlighted in bold font.
RAF, risk allele frequency; nsCL/P, non-syndromic cleft lip with or without cleft palate.

The sub-phenotype analysis revealed that the most significant SNVs identified in the present study were exclusively associated with the risk of nsCLP. No significant association was identified between them and the nsCLO risk (p_{trend} values > 0.05), however, the differences in ORs between nsCLP and nsCLO subgroups were not statistically significant (heterogeneity p-values >0.05). Besides, for all these variants, the trend test p-values for nsCLP were higher than the p-values for the overall phenotype (**Table 4**). Logistic regression analysis revealed that ARHGAP29 locus variants did not show evidence of gender-dependent association with the risk of nsCL/P. No significant sex genotype interactions were detected (**Table 5**).

Table 4. Association results for oral cleft sub-phenotypes

			RAF		
rs number	Alleles[a]	p_{trend}[b]	Patients	CControls	OR (95%CI)[d]
nsCLP					
rs3789688	T / C	2.48E-03	0.59	0.50	1.41 (1.13–1.76)
rs11165101	A / C	2.62E-04	0.67	0.57	1.54 (1.23–1.93)
rs11165110	A / G	**2.23E-04**	0.67	0.57	1.55 (1.23–1.94)
rs2065971	A / C	3.25E-04	0.65	0.55	1.51 (1.21–1.89)
rs2391467	A / G	**4.28E-06**	0.58	0.46	1.67 (1.34–2.08)
nsCLO					
rs3789688	T / C	5.68E-02	0.62	0.50	1.58 (0.99–2.54)
rs11165101	A / C	1.53E-01	0.65	0.57	1.43 (0.88–2.31)
rs11165110	A / G	2.51E-01	0.64	0.57	1.33 (0.83–2.12)
rs2065971	A / C	2.22E-01	0.63	0.55	1.34 (0.84–2.14)
rs2391467	A / G	7.03E-02	0.56	0.46	1.51 (0.95–2.38)

[a] The p_{trend} values below 2.54E-04 (0.05 / 197 SNVs) were interpreted statistically significant
For all tested SNVs, there was no heterogeneity between oral cleft sub-phenotypes (heterogeneity p-values between 0.551 and 0.780).
nsCLP, non-syndromic cleft lip and palate
nsCLO, non-syndromic cleft lip only

Table 5. Gender-dependent interaction of the most significant variants at the *ARHGAP29* locus and nsCL/P

SNV	OR_{int}(95%CI)[a]	p[d]	OR_{males}(95%CI)[b]	p[d]	$OR_{females}$(95%CI)[c]	p[d]
rs3789688	0.94 (0.62–1.43)	7.77E-01	1.44 (1.09–1.91)	1.16E-02	1.36 (1.00–1.84)	4.94E-02
rs11165101	0.89 (0.58–1.37)	5.91E-01	1.56 (1.16–2.10)	3.40E-03	1.39 (1.02–1.89)	3.63E-02
rs11165110	0.93 (0.61–1.43)	7.52E-01	1.51 (1.13–2.03)	6.03E-03	1.41 (1.04–1.92)	4.80E-02
rs2065971	0.88 (0.58–1.36)	5.73E-01	1.54 (1.14–2.07)	4.32E-03	1.36 (1.00–1.86)	3.03E-02
rs2391467	1.12 (0.73–1.71)	6.05E-01	1.72 (1.28–2.31)	3.24E-04	1.54 (1.13–2.09)	7.95E-03

[a] Odds ratio for the gene x gender interaction.
[b] Odds ratio for the males.
[c] Odds ratio for the females.
[d] Based on logistic regression under the additive model.

Table 6. Missense variants identified in the *ARHGAP29* gene with the use of SNV microarray

			NsCL/P Cases		Controls		MAF			
rs number	Alleles[a]	Amino acid change[b]	Genotypes	MAF	Genotypes	MAF	ExAC[c]	SIFT[d]	PolyPhen[e]	Mutation Assessor[f]
rs1999272	T / C	p.Gly1255Asp	0 / 1 / 268	0.002	0 / 0 / 569	0.000	0.0004	tolerated low confidence	benign	predicted non-functional (neutral)
rs143877998	T / G	p.Gln893Pro	0 / 1 / 268	0.002	0 / 0 / 569	0.000	0.0002	tolerated	benign	predicted functional (medium)
rs147752270	T / C	p.Val875Ile	0 / 1 / 268	0.002	0 / 2 / 567	0.002	0.0016	tolerated	benign	predicted non-functional (low)
rs41311172	T / C	p.Arg798Gln	0 / 0 / 269	0.000	0 / 3 / 566	0.003	0.0025	tolerated	probably damaging	predicted non-functional (low)
rs140877322	A / C	p.Arg348Leu	0 / 1 / 268	0.002	0 / 0 / 569	0.000	5.995E-05	deleterious	probably damaging	predicted functional (medium)
rs183410431	T / C	p.Arg84His	0 / 2 / 267	0.004	0 / 0 / 569	0.000	4.505E-05	deleterious	benign	predicted non-functional (neutral)

[a] Underline denotes the minor allele.
[b] ENST00000260526.11.
[c] Exome Aggregation Consortium (ExAC), European (Non-Finnish).
[d] http://genetics.bwh.harvard.edu/pph2/.
[e] http://sift.jcvi.org/.
[f] http://mutationassessor.org/r3/.

Missense variants

Six out of twenty-two (27.3%) genotyped missense *ARHGAP29* variants were identified in the tested samples. Four of them were found only in cleft patients, and one was found exclusively in controls (**Table 6**). One of the cleft specific variants (rs140877322, p. Arg348Leu) was predicted to be deleterious and functional by all prediction tools used in this study. In a non-Finnish European population of ExAC, the frequency of rs140877322 was 5.995E-05.

Discussion

Numerous genes and several polymorphic variants have been detected to confer an increased risk of nsCL/P [22,23]. Beaty et al. characterised four significant loci in GWAS for nsCL/P: *IRF6*, 8q24, *MAFB* (OMIM:*608968), and *ABCA4* [11], with multiple follow-up studies in different populations successfully confirming these loci [24–31]. A critical role for *IRF6* and the 8q24 region in craniofacial development has been previously identified, while the roles of the loci in the genes *ABCA4* and *MAFB* remains largely unclear [32–34]. The most significant SNVs strongly associated with nsCL/P have been identified within the introns of *ABCA4* [35]. However, *ABCA4* is not a good candidate as the etiologic gene for nsCL/P at the 1p22 locus because of its lack of expression in the developing lip or palate in mice [11]. Additionally, there were no reported defects in the craniofacial structure in mice homozygous for targeted loss-of-function mutations in *ABCA4* [36]. Moreover, despite identifying several missense mutations in *ABCA4* in humans, none of them showed suggestive evidence of causing craniofacial malformations [15,37]. However, a recent study suggests a role for *ARHGAP29* (a neighbouring gene of *ABCA4*) in nsCL/P based on expression in craniofacial development using a murine model. The *ARHGAP29* transcript was detected in the medial and lateral nasal processes, and expression was also observed in the mandibular and maxillary processes of developing mouse embryo at E10.5 and the shelves of the secondary palate at E13.5. [38]. Furthermore, its expression depends on *IRF6* [38], one of the pivotal contributors to the underlying genetics of human nsCLP/P [39]. *ARHGAP29* is located 47 kb centromeric to *ABCA4* and encodes Rho GTPase activating protein (GAP) 29, which is involved in many functions related to cellular shape, movement, proliferation, all essential for craniofacial development [12]. Rho is downstream of Tgfb and Wnt signalling pathways [40,41], which have also been implicated in craniofacial development. These are suggestive evidence that *ARHGAP29* is the etiologic gene at this locus and may play a role in nsCL/P. During the last few years, about twelve potentially pathogenic missense variants in *ARHGAP29* have been reported in nsCL/P cases. [17,19,28,35,42]. However, it is not clear if these possibly pathogenic rare variants contribute to the phenotype. Therefore, the purpose of this study was to evaluate the association between common and rare missense SNVs located within the *ARHGAP29* locus and the risk of nsCL/P in the Polish population. Patients with non-syndromic forms of cleft lip with cleft palate and cleft lip only were recruited, with patients with a diagnosis of non-syndromic cleft palate only were excluded from the molecular analyses due to the distinct aetiology of this subtype of oral clefts [43].

The findings showed that common nucleotide variants of the *ARHGAP29* gene are significantly correlated with the risk of nsCL/P. Statistical analysis of the genotyping results revealed that three common SNVs represent a single cleft association signal since they are located within the same intergenic region of *ARHGAP29/ABCD3* genes. These three risk variants rs11165101, rs11165110 and rs2391467 are strongly associated with the risk of this craniofacial anomaly in a tested group of patients. Our data also demonstrated that the minor allele carriers at rs2391467 have a 1.64-fold increased risk of nsCL/P. Moreover, all these results were statistically significant even after applying Bonferroni correction for multiple comparisons. Two other tested variants, rs3789688 and rs2065971, were close to reaching the study significance level. It is of note that the intronic SNV rs3789688 in families of non-Hispanic white ethnicities also showed a strong association with nsCL/P [29]. Although the intronic variants are unlikely to have effects on gene transcription or the final protein structure, multiple analysis revealed that non-coding variants have a significant role in the genetic causes of nsCL/P [3,35,44]. This suggests that the true casual variants implicated in the risk of nsCL/P might affect the *ARHGAP29* gene expression level rather than the structure of an encoded protein or are in pair-wise LD with an unknown actual pathogenic variant.

We hypothesised that some genetic risk for nsCL/P in Polish populations lies in rare exonic markers, thus, our study also included an analysis of twenty-two missense variants. Our results showed six missense *ARHGAP29* variants in the tested samples. One of the cleft specific variants (rs140877322, p.Arg348Leu) was predicted to be probably damaging and deleterious by multiple *in silico* tools. Furthermore, none of the unaffected individuals carried the variant. However, these results should be interpreted with caution because the functional impact of these variants is unknown. Therefore, functional studies in biological model systems are required to identify pathogenic variants and a possible mechanism contributing to the nsCL/P phenotype. Savastano at al. identified ten rare variants in the *ARHGAP29* gene using next-generation sequencing, of these, five were missense changes and the remaining were predicted to be loss-of-function (LoF). These findings provide evidence that the LoF variants but not missense variants may be an important genetic factor and contribute to the aetiology of nsCL/P [45]. To take this idea further, new coding variants which confer risk to nsCL/P should be identified by sequencing, which is crucial for rare variant discovery.

There was no evidence of a gender-dependent association with any of the SNVs studied. However, Carlson at al. confer that the impact of genetic variants on nsCL/P risk differs for males and females. These results are not surprising because the incidence rates of nsCL/P vary by sex. Carlson at al. used a genome-wide approach to identify the genetic contribution to this phenomenon and examined gene by sex interactions in a group of 2142 nsCL/P cases and 1700 controls recruited from different countries. Their analysis identified three loci that achieved genome-wide significance interaction effect, rs11142081, rs72804706, rs77590619 from the 9q22.1, 10q21.1, and 13q13.3 loci, respectively showed evidence of a higher risk of CL/P for females carrying the minor risk allele, while this trend was not present in males. It is worth noting that many biochemical mechanisms affect gene by sex interactions, hence they suggest that due to the diversity of possible mechanisms, it is challenging to explore or discuss each locus adequately [46].

Given the impact of rare variants as potential phenotypic modifiers diversity, which has been highlighted by Carlson at al. [47], we analysed cleft type-dependent association with the studied SNVs. However, our sub-phenotype analysis did not reveal any significant genotype-phenotype correlations. Nonetheless, these results should be interpreted with caution due to the small number of patients with nsCLO recruited to our study, therefore, insufficient power to detect significant cleft type differences.

The strength of this study lies in the homogenous study cohort recruited from a single ethnic group. Although the study is limited by a relatively small sample size, the risk variants with the lower allele frequencies may have been missed. Hence, statistical analyses were not well suited to draw reliable associations from low-frequency variants (MAF<0.05), which may be important in explaining nsCL/P susceptibility. Another limitation of our study is that the association analysis focused only on the genetic factors without considering environmental factors that appear to contribute to the aetiology of nsCL/P, like maternal folic acid supplementation or maternal smoking [48,49].

Despite these limitations, the nsCL/P risk loci identified in our research are consistent with previous studies and biological mechanisms, thereby providing further evidence for the role of *ARHGAP29* and new insight into the pathogenesis of the nsCL/P in the Polish population. Functional analyses are required to explore the mechanisms by which nucleotide variants of the *ARHGAP29* gene might increase risk of nsCL/P

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

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References

1. Mossey PA, Little J, Munger RG, Dixon MJ, Shaw WC. Cleft lip and palate. The Lancet. 2009 Nov;374(9703):1773-1785. https://doi.org/10.1016/s0140-6736(09)60695-4
2. Dixon MJ, Marazita ML, Beaty TH, Murray JC. Cleft lip and palate: understanding genetic and environmental influences. Nature Reviews Genetics. 2011 Feb 18;12(3):167-178. https://doi.org/10.1038/nrg2933

3. Leslie EJ, Marazita ML. Genetics of cleft lip and cleft palate. American Journal of Medical Genetics Part C: Seminars in Medical Genetics. 2013 Oct 4;163(4):246-258. https://doi.org/10.1002/ajmg.c.31381
4. Mangold E, Ludwig KU, Nöthen MM. Breakthroughs in the genetics of orofacial clefting. Trends in Molecular Medicine. 2011 Dec;17(12):725-733. https://doi.org/10.1016/j.molmed.2011.07.007
5. Murray J. Gene/environment causes of cleft lip and/or palate. Clinical Genetics. 2002 May 27;61(4):248-256. https://doi.org/10.1034/j.1399-0004.2002.610402.x
6. Beaty TH, Marazita ML, Leslie EJ. Genetic factors influencing risk to orofacial clefts: today's challenges and tomorrow's opportunities. F1000Research. 2016 Nov 30;5:2800. https://doi.org/10.12688/f1000research.9503.1
7. Leslie EJ, Carlson JC, Shaffer JR, Butali A, Buxó CJ, Castilla EE, Christensen K, Deleyiannis FWB, Leigh Field L, Hecht JT, Moreno L, Orioli IM, Padilla C, Vieira AR, Wehby GL, Feingold E, Weinberg SM, Murray JC, Beaty TH, Marazita ML. Genome-wide meta-analyses of nonsyndromic orofacial clefts identify novel associations between FOXE1 and all orofacial clefts, and TP63 and cleft lip with or without cleft palate. Human Genetics. 2017 Jan 4;136(3):275-286. https://doi.org/10.1007/s00439-016-1754-7
8. Ludwig KU, Böhmer AC, Bowes J, Nikolić M, Ishorst N, Wyatt N, Hammond NL, Gölz L, Thieme F, Barth S, Schuenke H, Klamt J, Spielmann M, Aldhorae K, Rojas-Martinez A, Nöthen MM, Rada-Iglesias A, Dixon MJ, Knapp M, Mangold E. Imputation of Orofacial Clefting Data Identifies Novel Risk Loci and Sheds Light on the Genetic Background of Cleft Lip ± Cleft Palate and Cleft Palate Only.. Human Molecular Genetics. 2017 Jan 13;:ddx012. https://doi.org/10.1093/hmg/ddx012
9. Mostowska A, Gaczkowska A, Żukowski K, Ludwig K, Hozyasz K, Wójcicki P, Mangold E, Böhmer A, Heilmann-Heimbach S, Knapp M, Zadurska M, Biedziak B, Budner M, Lasota A, Daktera-Micker A, Jagodziński P. Common variants in DLG1 locus are associated with non-syndromic cleft lip with or without cleft palate. Clinical Genetics. 2018 Feb 11;93(4):784-793. https://doi.org/10.1111/cge.13141
10. Yu Y, Zuo X, He M, Gao J, Fu Y, Qin C, Meng L, Wang W, Song Y, Cheng Y, Zhou F, Chen G, Zheng X, Wang X, Liang B, Zhu Z, Fu X, Sheng Y, Hao J, Liu Z, Yan H, Mangold E, Ruczinski I, Liu J, Marazita ML, Ludwig KU, Beaty TH, Zhang X, Sun L, Bian Z. Genome-wide analyses of non-syndromic cleft lip with palate identify 14 novel loci and genetic heterogeneity. Nature Communications. 2017 Feb 24;8(1). https://doi.org/10.1038/ncomms14364
11. Beaty TH, Murray JC, Marazita ML, Munger RG, Ruczinski I, Hetmanski JB, Liang KY, Wu T, Murray T, Fallin MD, Redett RA, Raymond G, Schwender H, Jin S, Cooper ME, Dunnwald M, Mansilla MA, Leslie E, Bullard S, Lidral AC, Moreno LM, Menezes R, Vieira AR, Petrin A, Wilcox AJ, Lie RT, Jabs EW, Wu-Chou YH, Chen PK, Wang H, Ye X, Huang S, Yeow V, Chong SS, Jee SH, Shi B, Christensen K, Melbye M, Doheny KF, Pugh EW, Ling H, Castilla EE, Czeizel AE, Ma L, Field LL, Brody L, Pangilinan F, Mills JL, Molloy AM, Kirke PN, Scott JM, Arcos-Burgos M, Scott AF. A genome-wide association study of cleft lip with and without cleft palate identifies risk variants near MAFB and ABCA4. Nature Genetics. 2010 May 2;42(6):525-529. https://doi.org/10.1038/ng.580
12. Birnbaum S, Ludwig KU, Reutter H, Herms S, Steffens M, Rubini M, Baluardo C, Ferrian M, Almeida de Assis N, Alblas MA, Barth S, Freudenberg J, Lauster C, Schmidt G, Scheer M, Braumann B, Bergé SJ, Reich RH, Schiefke F, Hemprich A, Pötzsch S, Steegers-Theunissen RP, Pötzsch B, Moebus S, Horsthemke B, Kramer F, Wienker TF, Mossey PA, Propping P, Cichon S, Hoffmann P, Knapp M, Nöthen MM, Mangold E. Key susceptibility locus for nonsyndromic cleft lip with or without cleft palate on chromosome 8q24. Nature Genetics. 2009 Mar 8;41(4):473-477. https://doi.org/10.1038/ng.333
13. Grant SF, Wang K, Zhang H, Glaberson W, Annaiah K, Kim CE, Bradfield JP, Glessner JT, Thomas KA, Garris M, Frackelton EC, Otieno FG, Chiavacci RM, Nah H, Kirschner RE, Hakonarson H. A Genome-Wide Association Study Identifies a Locus for Nonsyndromic Cleft Lip with or without Cleft Palate on 8q24. The Journal of Pediatrics. 2009 Dec;155(6):909-913. https://doi.org/10.1016/j.jpeds.2009.06.020
14. Mangold E, Ludwig KU, Birnbaum S, Baluardo C, Ferrian M, Herms S, Reutter H, de Assis NA, Chawa TA, Mattheisen M, Steffens M, Barth S, Kluck N, Paul A, Becker J, Lauster C, Schmidt G, Braumann B, Scheer M, Reich RH, Hemprich A, Pötzsch S, Blaumeiser B, Moebus S, Krawczak M, Schreiber S, Meitinger T, Wichmann H, Steegers-Theunissen RP, Kramer F, Cichon S, Propping P, Wienker TF, Knapp M, Rubini M, Mossey PA, Hoffmann P, Nöthen MM. Genome-wide association study identifies two susceptibility loci for nonsyndromic cleft lip with or without cleft palate. Nature Genetics. 2009 Dec 20;42(1):24-26. https://doi.org/10.1038/ng.506
15. Beaty TH, Murray JC, Marazita ML, Munger RG, Ruczinski I, Hetmanski JB, Liang KY, Wu T, Murray T, Fallin MD, Redett RA, Raymond G, Schwender H, Jin S, Cooper ME, Dunnwald M, Mansilla MA, Leslie E, Bullard S, Lidral AC, Moreno LM, Menezes R, Vieira AR, Petrin A, Wilcox AJ, Lie RT, Jabs EW, Wu-Chou YH, Chen PK, Wang H, Ye X, Huang S, Yeow V, Chong SS, Jee SH, Shi B, Christensen K, Melbye M, Doheny KF, Pugh EW, Ling H, Castilla EE, Czeizel AE, Ma L, Field LL, Brody L, Pangilinan F, Mills JL, Molloy AM, Kirke PN, Scott JM, Arcos-Burgos M, Scott AF. A genome-wide association study of cleft lip with and without cleft palate identifies risk variants near MAFB and ABCA4. Nature Genetics. 2010 May 2;42(6):525-529. https://doi.org/10.1038/ng.580
16. Burke TR, Tsang SH. Allelic and phenotypic heterogeneity inABCA4mutations. Ophthalmic Genetics. 2011 Apr 21;32(3):165-174. https://doi.org/10.3109/13816810.2011.565397
17. Leslie EJ, Mansilla MA, Biggs LC, Schuette K, Bullard S, Cooper M, Dunnwald M, Lidral AC, Marazita ML, Beaty TH, Murray JC. Expression and mutation analyses implicate ARHGAP29 as the etiologic gene for the cleft lip with or without cleft palate locus identified by genome-wide association on chromosome

1p22. Birth Defects Research Part A: Clinical and Molecular Teratology. 2012 Sep 24;94(11):934-942. https://doi.org/10.1002/bdra.23076
18. Biggs LC, Naridze RL, DeMali KA, Lusche DF, Kuhl S, Soll DR, Schutte BC, Dunnwald M. Interferon regulatory factor 6 regulates keratinocyte migration. Journal of Cell Science. 2014 Apr 28;127(13):2840-2848. https://doi.org/10.1242/jcs.139246
19. Liu H, Busch T, Eliason S, Anand D, Bullard S, Gowans LJ, Nidey N, Petrin A, Augustine-Akpan E, Saadi I, Dunnwald M, Lachke SA, Zhu Y, Adeyemo A, Amendt B, Roscioli T, Cornell R, Murray J, Butali A. Exome sequencing provides additional evidence for the involvement ofARHGAP29in Mendelian orofacial clefting and extends the phenotypic spectrum to isolated cleft palate. Birth Defects Research. 2017 Jan 20;109(1):27-37. https://doi.org/10.1002/bdra.23596
20. Gaczkowska A, Żukowski K, Biedziak B, Hozyasz KK, Wójcicki P, Zadurska M, Budner M, Lasota A, Szponar-Żurowska A, Jagodziński PP, Mostowska A. Association of CDKAL1 nucleotide variants with the risk of non-syndromic cleft lip with or without cleft palate. Journal of Human Genetics. 2018 Feb 5;63(4):397-406. https://doi.org/10.1038/s10038-017-0397-4
21. Mostowska A, Hozyasz KK, Wójcicki P, Biedziak B, Wesoły J, Sowińska A, Matuszewska-Trojan S, Jagodziński PP. Searching for new genes and loci involved in cleft lip and palate in the Polish population – genome-wide association study. Journal of Medical Science. 2016 Feb;83(3):265-8. https://doi.org/10.20883/medical.78
22. Ludwig KU, Mangold E, Herms S, Nowak S, Reutter H, Paul A, Becker J, Herberz R, AlChawa T, Nasser E, Böhmer AC, Mattheisen M, Alblas MA, Barth S, Kluck N, Lauster C, Braumann B, Reich RH, Hemprich A, Pötzsch S, Blaumeiser B, Daratsianos N, Kreusch T, Murray JC, Marazita ML, Ruczinski I, Scott AF, Beaty TH, Kramer F, Wienker TF, Steegers-Theunissen RP, Rubini M, Mossey PA, Hoffmann P, Lange C, Cichon S, Propping P, Knapp M, Nöthen MM. Genome-wide meta-analyses of nonsyndromic cleft lip with or without cleft palate identify six new risk loci. Nature Genetics. 2012 Aug 5;44(9):968-971. https://doi.org/10.1038/ng.2360
23. Mangold E, Ludwig KU, Birnbaum S, Baluardo C, Ferrian M, Herms S, Reutter H, de Assis NA, Chawa TA, Mattheisen M, Steffens M, Barth S, Kluck N, Paul A, Becker J, Lauster C, Schmidt G, Braumann B, Scheer M, Reich RH, Hemprich A, Pötzsch S, Blaumeiser B, Moebus S, Krawczak M, Schreiber S, Meitinger T, Wichmann H, Steegers-Theunissen RP, Kramer F, Cichon S, Propping P, Wienker TF, Knapp M, Rubini M, Mossey PA, Hoffmann P, Nöthen MM. Genome-wide association study identifies two susceptibility loci for nonsyndromic cleft lip with or without cleft palate. Nature Genetics. 2009 Dec 20;42(1):24-26. https://doi.org/10.1038/ng.506
24. Yuan Q, Blanton SH, Hecht JT. Association of ABCA4 and MAFB with non-syndromic cleft lip with or without cleft palate. American Journal of Medical Genetics Part A. 2011 May 12;155(6):1469-1471. https://doi.org/10.1002/ajmg.a.33940
25. Fontoura C, Silva RM, Granjeiro JM, Letra A. Further evidence of association of theABCA4gene with cleft lip/palate. European Journal of Oral Sciences. 2012 Oct 15;120(6):553-557. https://doi.org/10.1111/eos.12001
26. Lennon CJ, Birkeland AC, Nuñez JAP, Su GH, Lanzano P, Guzman E, Celis K, Eisig SB, Hoffman D, Rendon MTG, Ostos H, Chung WK, Haddad J. Association of candidate genes with nonsyndromic clefts in Honduran and Colombian populations. The Laryngoscope. 2012 Jul 2;122(9):2082-2087. https://doi.org/10.1002/lary.23394
27. Beaty TH, Taub MA, Scott AF, Murray JC, Marazita ML, Schwender H, Parker MM, Hetmanski JB, Balakrishnan P, Mansilla MA, Mangold E, Ludwig KU, Noethen MM, Rubini M, Elcioglu N, Ruczinski I. Confirming genes influencing risk to cleft lip with/without cleft palate in a case–parent trio study. Human Genetics. 2013 Jul 20;132(7):771-781. https://doi.org/10.1007/s00439-013-1283-6
28. Butali A, Mossey P, Adeyemo W, Eshete M, Gaines L, Braimah R, Aregbesola B, Rigdon J, Emeka C, Olutayo J, Ogunlewe O, Ladeinde A, Abate F, Hailu T, Mohammed I, Gravem P, Deribew M, Gesses M, Adeyemo A, Marazita M, Murray J. Rare functional variants in genome-wide association identified candidate genes for nonsyndromic clefts in the African population. American Journal of Medical Genetics Part A. 2014 Jul 31;164(10):2567-2571. https://doi.org/10.1002/ajmg.a.36691
29. Letra A, Maili L, Mulliken JB, Buchanan E, Blanton SH, Hecht JT. Further evidence suggesting a role for variation inARHGAP29variants in nonsyndromic cleft lip/palate. Birth Defects Research Part A: Clinical and Molecular Teratology. 2014 Aug 27;100(9):679-685. https://doi.org/10.1002/bdra.23286
30. Gowans L, Adeyemo W, Eshete M, Mossey P, Busch T, Aregbesola B, Donkor P, Arthur F, Bello S, Martinez A, Li M, Augustine-Akpan E, Deressa W, Twumasi P, Olutayo J, Deribew M, Agbenorku P, Oti A, Braimah R, Plange-Rhule G, Gesses M, Obiri-Yeboah S, Oseni G, Olaitan P, Abdur-Rahman L, Abate F, Hailu T, Gravem P, Ogunlewe M, Buxó C, Marazita M, Adeyemo A, Murray J, Butali A. Association Studies and Direct DNA Sequencing Implicate Genetic Susceptibility Loci in the Etiology of Nonsyndromic Orofacial Clefts in Sub-Saharan African Populations. Journal of Dental Research. 2016 Jul 20;95(11):1245-1256. https://doi.org/10.1177/0022034516657003
31. Leslie EJ, Carlson JC, Shaffer JR, Feingold E, Wehby G, Laurie CA, Jain D, Laurie CC, Doheny KF, McHenry T, Resick J, Sanchez C, Jacobs J, Emanuele B, Vieira AR, Neiswanger K, Lidral AC, Valencia-Ramirez LC, Lopez-Palacio AM, Valencia DR, Arcos-Burgos M, Czeizel AE, Field LL, Padilla CD, Cutiongco-de la Paz EMC, Deleyiannis F, Christensen K, Munger RG, Lie RT, Wilcox A, Romitti PA, Castilla EE, Mereb JC, Poletta FA, Orioli IM, Carvalho FM, Hecht JT, Blanton SH, Buxó CJ, Butali A, Mossey PA, Adeyemo WL, James O, Braimah RO, Aregbesola BS, Eshete MA, Abate F, Koruyucu M, Seymen F, Ma L, de Salamanca JE, Weinberg SM, Moreno L, Murray JC, Marazita ML. A multi-ethnic genome-wide association study

identifies novel loci for non-syndromic cleft lip with or without cleft palate on 2p24.2, 17q23 and 19q13. Human Molecular Genetics. 2016 Mar 30;:ddw104. https://doi.org/10.1093/hmg/ddw104
32. Kondo S, Schutte BC, Richardson RJ, Bjork BC, Knight AS, Watanabe Y, Howard E, Ferreira de Lima RL, Daack-Hirsch S, Sander A, McDonald-McGinn DM, Zackai EH, Lammer EJ, Aylsworth AS, Ardinger HH, Lidral AC, Pober BR, Moreno L, Arcos-Burgos M, Valencia C, Houdayer C, Bahuau M, Moretti-Ferreira D, Richieri-Costa A, Dixon MJ, Murray JC. Mutations in IRF6 cause Van der Woude and popliteal pterygium syndromes. Nature Genetics. 2002 Sep 3;32(2):285-289. https://doi.org/10.1038/ng985
33. Uslu VV, Petretich M, Ruf S, Langenfeld K, Fonseca NA, Marioni JC, Spitz F. Long-range enhancers regulating Myc expression are required for normal facial morphogenesis. Nature Genetics. 2014 May 25;46(7):753-758. https://doi.org/10.1038/ng.2971
34. Paul B, Palmer K, Sharp J, Pratt C, Murray S, Dunnwald M. ARHGAP29 Mutation Is Associated with Abnormal Oral Epithelial Adhesions. Journal of Dental Research. 2017 Aug 17;96(11):1298-1305. https://doi.org/10.1177/0022034517726079
35. Leslie E, Taub M, Liu H, Steinberg K, Koboldt D, Zhang Q, Carlson J, Hetmanski J, Wang H, Larson D, Fulton R, Kousa Y, Fakhouri W, Naji A, Ruczinski I, Begum F, Parker M, Busch T, Standley J, Rigdon J, Hecht J, Scott A, Wehby G, Christensen K, Czeizel A, Deleyiannis F, Schutte B, Wilson R, Cornell R, Lidral A, Weinstock G, Beaty T, Marazita M, Murray J. Identification of Functional Variants for Cleft Lip with or without Cleft Palate in or near PAX7, FGFR2, and NOG by Targeted Sequencing of GWAS Loci. The American Journal of Human Genetics. 2015 Mar;96(3):397-411. https://doi.org/10.1016/j.ajhg.2015.01.004
36. Weng J, Mata NL, Azarian SM, Tzekov RT, Birch DG, Travis GH. Insights into the Function of Rim Protein in Photoreceptors and Etiology of Stargardt's Disease from the Phenotype in abcr Knockout Mice. Cell. 1999 Jul;98(1):13-23. https://doi.org/10.1016/s0092-8674(00)80602-9
37. Burke TR, Tsang SH. Allelic and phenotypic heterogeneity inABCA4mutations. Ophthalmic Genetics. 2011 Apr 21;32(3):165-174. https://doi.org/10.3109/13816810.2011.565397
38. Leslie EJ, Mansilla MA, Biggs LC, Schuette K, Bullard S, Cooper M, Dunnwald M, Lidral AC, Marazita ML, Beaty TH, Murray JC. Expression and mutation analyses implicate ARHGAP29 as the etiologic gene for the cleft lip with or without cleft palate locus identified by genome-wide association on chromosome 1p22. Birth Defects Research Part A: Clinical and Molecular Teratology. 2012 Sep 24;94(11):934-942. https://doi.org/10.1002/bdra.23076
39. Zucchero TM, Cooper ME, Maher BS, Daack-Hirsch S, Nepomuceno B, Ribeiro L, Caprau D, Christensen K, Suzuki Y, Machida J, Natsume N, Yoshiura K, Vieira AR, Orioli IM, Castilla EE, Moreno L, Arcos-Burgos M, Lidral AC, Field LL, Liu Y, Ray A, Goldstein TH, Schultz RE, Shi M, Johnson MK, Kondo S, Schutte BC, Marazita ML, Murray JC. Interferon Regulatory Factor 6 (IRF6) Gene Variants and the Risk of Isolated Cleft Lip or Palate. New England Journal of Medicine. 2004 Aug 19;351(8):769-780. https://doi.org/10.1056/nejmoa032909
40. Kardassis D, Murphy C, Fotsis T, Moustakas A, Stournaras C. Control of transforming growth factor β signal transduction by small GTPases. FEBS Journal. 2009 Jun;276(11):2947-2965. https://doi.org/10.1111/j.1742-4658.2009.07031.x
41. Schlessinger K, Hall A, Tolwinski N. Wnt signaling pathways meet Rho GTPases. Genes & Development. 2009 Feb 1;23(3):265-277. https://doi.org/10.1101/gad.1760809
42. Chandrasekharan D, Ramanathan A. Identification of a novel heterozygous truncation mutation in exon 1 of ARHGAP29 in an Indian subject with non-syndromic cleft lip with cleft palate. European Journal of Dentistry. 2014 Oct;8(4):528-532. https://doi.org/10.4103/1305-7456.143637
43. Wen Y, Lu Q. Risk prediction models for oral clefts allowing for phenotypic heterogeneity. Frontiers in Genetics. 2015 Aug 13;6. https://doi.org/10.3389/fgene.2015.00264
44. Rahimov F, Marazita ML, Visel A, Cooper ME, Hitchler MJ, Rubini M, Domann FE, Govil M, Christensen K, Bille C, Melbye M, Jugessur A, Lie RT, Wilcox AJ, Fitzpatrick DR, Green ED, Mossey PA, Little J, Steegers-Theunissen RP, Pennacchio LA, Schutte BC, Murray JC. Disruption of an AP-2α binding site in an IRF6 enhancer is associated with cleft lip. Nature Genetics. 2008 Oct 5;40(11):1341-1347. https://doi.org/10.1038/ng.242
45. Savastano C, Brito L, Faria Á, Setó-Salvia N, Peskett E, Musso C, Alvizi L, Ezquina S, James C, GOSgene, Beales P, Lees M, Moore G, Stanier P, Passos-Bueno M. Impact of rare variants inARHGAP29to the etiology of oral clefts: role of loss-of-functionvsmissense variants. Clinical Genetics. 2016 Jul 26;91(5):683-689. https://doi.org/10.1111/cge.12823
46. Carlson JC, Nidey NL, Butali A, Buxo CJ, Christensen K, Deleyiannis FW, Hecht JT, Field LL, Moreno-Uribe LM, Orioli IM, Poletta FA, Padilla C, Vieira AR, Weinberg SM, Wehby GL, Feingold E, Murray JC, Marazita ML, Leslie EJ. Genome-wide interaction studies identify sex-specific risk alleles for nonsyndromic orofacial clefts. Genetic Epidemiology. 2018 Sep 11;42(7):664-672. https://doi.org/10.1002/gepi.22158
47. Carlson JC, Taub MA, Feingold E, Beaty TH, Murray JC, Marazita ML, Leslie EJ. Identifying Genetic Sources of Phenotypic Heterogeneity in Orofacial Clefts by Targeted Sequencing. Birth Defects Research. 2017 Aug 1;109(13):1030-1038. https://doi.org/10.1002/bdr2.23605
48. Wehby G, Murray J. Folic acid and orofacial clefts: a review of the evidence. Oral Diseases. 2010 Jan;16(1):11-19. https://doi.org/10.1111/j.1601-0825.2009.01587.x
49. Hackshaw A, Rodeck C, Boniface S. Maternal smoking in pregnancy and birth defects: a systematic review based on 173 687 malformed cases and 11.7 million controls. Human Reproduction Update. 2011 Jul 11;17(5):589-604. https://doi.org/10.1093/humupd/dmr022

Supplementary Table 1. Association results for common nucleotide variants at the *ARHGAP29* locusa.

rs number	Location (bp)[b]	Gene	Exonic variant	Alleles[c]	MAF[d]	P_{trend}-value
rs743117	chr1: 94441950			T / c	0.37	1.12E-01
rs743113	chr1: 94442739			T / c	0.41	2.20E-01
rs1889547	chr1: 94450131			A / g	0.44	1.31E-01
rs11165057	chr1: 94455420			t / G	0.08	1.72E-01
rs11165058	chr1: 94455721			t / G	0.08	1.42E-01
rs17110736	chr1: 94462769	ABCA4		a / G	0.07	7.88E-01
rs3789375	chr1: 94465132	ABCA4		T / g	0.11	4.69E-01
rs4147871	chr1: 94465461	ABCA4		A / g	0.06	7.72E-01
rs4147869	chr1: 94465677	ABCA4		t / C	0.06	5.75E-01
rs4147868	chr1: 94466066	ABCA4		g / C	0.06	6.62E-01
rs12070273	chr1: 94466088	ABCA4		A / g	0.06	6.62E-01
rs4147864	chr1: 94467238	ABCA4		a / G	0.06	5.70E-01
rs17110761	chr1: 94467407	ABCA4		t / C	0.06	6.62E-01
rs7537325	chr1: 94469631	ABCA4		T / c	0.23	9.20E-01
rs1762114	chr1: 94471075	ABCA4	Synonymous_I2023I	a / G	0.07	9.80E-01
rs4147863	chr1: 94471154	ABCA4		t / C	0.18	6.29E-01
rs4147862	chr1: 94471519	ABCA4		t / C	0.18	8.63E-01
rs4147861	chr1: 94471948	ABCA4		a / C	0.06	5.86E-01
rs557026	chr1: 94472489	ABCA4		t / C	0.11	2.43E-01
rs3789379	chr1: 94472520	ABCA4		A / g	0.18	8.63E-01
rs7531001	chr1: 94472909	ABCA4		a / G	0.18	6.54E-01
rs2275029	chr1: 94473845	ABCA4	Synonymous_P1948P	T / c	0.16	7.95E-01
rs2275031	chr1: 94473896	ABCA4		t / G	0.18	7.89E-01
rs1191234	chr1: 94474020	ABCA4		A / g	0.11	2.21E-01
rs2275032	chr1: 94474185	ABCA4		A / c	0.18	8.95E-01
rs4147857	chr1: 94474328	ABCA4	Synonymous_L1938L	T / c	0.19	5.82E-01
rs4147856	chr1: 94474452	ABCA4		T / g	0.19	5.78E-01
rs17110808	chr1: 94474872	ABCA4		a / G	0.07	2.41E-01
rs2065712	chr1: 94476035	ABCA4		t / C	0.25	6.17E-01
rs11165062	chr1: 94477634	ABCA4		a / G	0.13	5.36E-01
rs945067	chr1: 94477893	ABCA4		t / C	0.20	9.69E-01
rs17391542	chr1: 94477935	ABCA4		a / G	0.11	6.99E-01
rs12085639	chr1: 94478293	ABCA4		a / G	0.16	9.93E-01
rs486879	chr1: 94478425	ABCA4		T / c	0.20	7.67E-01
rs567370	chr1: 94478573	ABCA4		T / c	0.33	9.75E-01
rs12082181	chr1: 94478595	ABCA4		T / c	0.33	8.37E-01
rs17391612	chr1: 94478847	ABCA4		a / G	0.12	5.87E-01
rs3789391	chr1: 94479338	ABCA4		t / C	0.06	6.29E-01
rs12049183	chr1: 94479468	ABCA4		t / C	0.06	5.89E-01
rs2275034	chr1: 94480439	ABCA4		t / C	0.40	5.85E-01
rs3818778	chr1: 94480529	ABCA4		a / C	0.48	1.52E-01
rs914958	chr1: 94481068	ABCA4		A / g	0.21	7.47E-01
rs915201	chr1: 94481596	ABCA4		A / g	0.25	9.13E-01

rs number	Location (bp)[b]	Gene	Exonic variant	Alleles[c]	MAF[d]	P_{trend}-value
rs915200	chr1: 94481904	ABCA4		t / C	0.08	7.70E-01
rs915199	chr1: 94481929	ABCA4		t / C	0.23	8.72E-01
rs6681968	chr1: 94484705	ABCA4		t / C	0.42	4.56E-01
rs17110850	chr1: 94485491	ABCA4		A / g	0.11	6.34E-01
rs10493867	chr1: 94486406	ABCA4		T / c	0.25	8.25E-01
rs4147848	chr1: 94486587	ABCA4		t / G	0.29	4.45E-01
rs933073	chr1: 94486667	ABCA4		T / g	0.08	1.62E-01
rs472908	chr1: 94487354	ABCA4		A / g	0.43	1.59E-01
rs2282229	chr1: 94488326	ABCA4		a / T	0.09	1.96E-01
rs1932014	chr1: 94488497	ABCA4		A / g	0.44	3.67E-01
rs1889407	chr1: 94489553	ABCA4		T / g	0.44	3.49E-01
rs2151847	chr1: 94489975	ABCA4		a / C	0.43	4.86E-01
rs11165065	chr1: 94491468	ABCA4		a / G	0.32	5.83E-01
rs3945204	chr1: 94492773	ABCA4		t / C	0.44	5.79E-01
rs4147846	chr1: 94495407	ABCA4		T / c	0.49	6.68E-01
rs4147845	chr1: 94495417	ABCA4		T / c	0.49	6.69E-01
rs4147844	chr1: 94495487	ABCA4		A / g	0.49	6.69E-01
rs2297671	chr1: 94496253	ABCA4		A / g	0.50	6.78E-01
rs4147841	chr1: 94497178	ABCA4		A / g	0.44	4.42E-01
rs3789393	chr1: 94499133	ABCA4		t / C	0.44	4.39E-01
rs3789395	chr1: 94501594	ABCA4		a / C	0.43	3.33E-01
rs1320502	chr1: 94501799	ABCA4		T / c	0.43	3.89E-01
rs1889548	chr1: 94503197	ABCA4		a / C	0.31	7.80E-01
rs11165069	chr1: 94504545	ABCA4		t / C	0.21	8.74E-01
rs2297633	chr1: 94510673	ABCA4		t / G	0.34	6.51E-01
rs3789399	chr1: 94511717	ABCA4		c / G	0.48	2.89E-01
rs544830	chr1: 94512893	ABCA4		T / c	0.48	4.56E-01
rs4147836	chr1: 94516474	ABCA4		t / C	0.22	9.65E-02
rs1191231	chr1: 94516985	ABCA4		a / C	0.48	4.73E-01
rs497511	chr1: 94523113	ABCA4		A / g	0.46	5.19E-01
rs549848	chr1: 94524856	ABCA4		T / c	0.34	4.39E-01
rs521538	chr1: 94525623	ABCA4		a / G	0.22	5.97E-01
rs4147833	chr1: 94528363	ABCA4		t / C	0.21	5.94E-02
rs4847273	chr1: 94529743	ABCA4		A / g	0.26	7.51E-02
rs1007347	chr1: 94530518	ABCA4		T / c	0.26	7.36E-02
rs553608	chr1: 94531013	ABCA4		t / C	0.21	2.17E-01
rs1191232	chr1: 94531192	ABCA4		a / G	0.37	3.28E-01
rs3789405	chr1: 94531324	ABCA4		T / c	0.26	6.85E-02
rs3789407	chr1: 94531606	ABCA4		c / G	0.22	1.08E-01
rs4140392	chr1: 94532013	ABCA4		t / C	0.21	6.51E-02
rs1191228	chr1: 94532562	ABCA4		t / C	0.21	2.17E-01
rs1931575	chr1: 94533014	ABCA4		T / c	0.23	2.76E-01
rs549114	chr1: 94534354	ABCA4		a / G	0.33	7.45E-01
rs2151849	chr1: 94535174	ABCA4		A / g	0.24	3.28E-01

rs number	Location (bp)[b]	Gene	Exonic variant	Alleles[c]	MAF[d]	P_{trend}-value
rs3789411	chr1: 94535546	ABCA4		a / G	0.33	8.01E-01
rs4612636	chr1: 94535689	ABCA4		A / c	0.06	7.91E-01
rs3789412	chr1: 94536067	ABCA4		t / C	0.24	2.06E-01
rs12758774	chr1: 94537295	ABCA4		t / C	0.20	9.00E-01
rs12759306	chr1: 94537642	ABCA4		a / C	0.20	9.17E-01
rs1761375	chr1: 94538011	ABCA4		a / G	0.31	9.04E-01
rs492220	chr1: 94542569	ABCA4		T / c	0.29	6.85E-01
rs3120133	chr1: 94542770	ABCA4		T / g	0.08	5.64E-01
rs4147831	chr1: 94544233	ABCA4	Synonymous_H423H	a / G	0.07	3.45E-01
rs4147828	chr1: 94547889	ABCA4		A / g	0.18	8.39E-02
rs4147827	chr1: 94548080	ABCA4		G / c	0.23	1.50E-02
rs574741	chr1: 94549083	ABCA4		t / C	0.22	6.14E-01
rs546550	chr1: 94550555	ABCA4		A / g	0.29	7.35E-01
rs17461953	chr1: 94551450	ABCA4		A / c	0.23	1.89E-02
rs563429	chr1: 94553866	ABCA4		A / g	0.29	6.51E-01
rs4847196	chr1: 94554453	ABCA4		a / G	0.22	1.06E-01
rs1191238	chr1: 94556894	ABCA4		a / G	0.09	8.84E-01
rs554931	chr1: 94557357	ABCA4		t / C	0.23	7.85E-01
rs483904	chr1: 94557434	ABCA4		t / C	0.23	7.85E-01
rs952499	chr1: 94558425	ABCA4		T / c	0.47	2.95E-01
rs538880	chr1: 94558774	ABCA4		g / C	0.23	8.19E-01
rs2068334	chr1: 94559715	ABCA4		a / G	0.17	2.25E-01
rs4147825	chr1: 94560938	ABCA4		a / G	0.38	8.42E-01
rs4147823	chr1: 94561272	ABCA4		A / c	0.22	6.95E-01
rs4147820	chr1: 94562084	ABCA4		t / C	0.21	6.05E-01
rs12088309	chr1: 94563916	ABCA4		T / c	0.30	1.14E-01
rs3789421	chr1: 94565577	ABCA4		a / G	0.19	8.61E-01
rs950283	chr1: 94567223	ABCA4		T / c	0.36	6.74E-03
rs4147819	chr1: 94569504	ABCA4		a / G	0.08	2.29E-01
rs481931	chr1: 94570016	ABCA4		t / G	0.39	1.25E-03
rs570926	chr1: 94570218	ABCA4		T / c	0.39	1.02E-03
rs570878	chr1: 94570234	ABCA4		t / G	0.48	1.93E-02
rs1211213	chr1: 94571420	ABCA4		A / g	0.36	1.90E-03
rs4147816	chr1: 94574780	ABCA4		t / C	0.40	2.56E-03
rs4147812	chr1: 94575043	ABCA4		A / c	0.40	2.50E-03
rs3827712	chr1: 94575171	ABCA4		T / c	0.40	3.37E-03
rs3789433	chr1: 94575440	ABCA4		a / G	0.26	2.87E-01
rs3789434	chr1: 94575978	ABCA4		T / c	0.08	2.52E-01
rs3789435	chr1: 94576360	ABCA4		A / g	0.48	2.09E-02
rs3827713	chr1: 94576524	ABCA4		c / G	0.48	2.09E-02
rs4147810	chr1: 94576664	ABCA4		A / g	0.08	2.52E-01
rs2297635	chr1: 94576893	ABCA4		a / G	0.08	3.08E-01
rs2297634	chr1: 94576968	ABCA4		T / c	0.48	2.09E-02
rs1889405	chr1: 94577410	ABCA4		t / C	0.24	3.26E-01

rs number	Location (bp)[b]	Gene	Exonic variant	Alleles[c]	MAF[d]	P_{trend}-value
rs1889404	chr1: 94577423	ABCA4		t / C	0.24	2.67E-01
rs3789438	chr1: 94577462	ABCA4		t / G	0.08	2.98E-01
rs4147807	chr1: 94579053	ABCA4		A / g	0.22	4.89E-01
rs3789439	chr1: 94579426	ABCA4		T / c	0.22	2.56E-01
rs10782976	chr1: 94581125	ABCA4		a / G	0.31	3.96E-01
rs3789441	chr1: 94581384	ABCA4		t / C	0.27	2.56E-01
rs3789442	chr1: 94581456	ABCA4		c / G	0.22	2.14E-01
rs3789443	chr1: 94581529	ABCA4		A / g	0.23	2.06E-01
rs3789444	chr1: 94581540	ABCA4		t / C	0.23	1.84E-01
rs7535005	chr1: 94581905	ABCA4		t / C	0.25	3.63E-01
rs3789445	chr1: 94582249	ABCA4		T / g	0.25	1.08E-01
rs4147803	chr1: 94582293	ABCA4		g / C	0.43	9.24E-02
rs4147798	chr1: 94585009	ABCA4		t / C	0.23	1.60E-01
rs3761906	chr1: 94587362			t / G	0.05	7.37E-01
rs2151846	chr1: 94587687			T / g	0.43	1.06E-01
rs1931572	chr1: 94588992			T / c	0.40	5.48E-02
rs10874835	chr1: 94591481			a / G	0.23	2.55E-01
rs1105123	chr1: 94592290			T / c	0.28	1.40E-01
rs12071152	chr1: 94593399			a / G	0.05	9.04E-01
rs11802196	chr1: 94594043			A / c	0.41	8.50E-02
rs11165081	chr1: 94594080			A / c	0.41	8.00E-02
rs17111122	chr1: 94596464			A / g	0.25	1.59E-01
rs6686599	chr1: 94596831			a / G	0.39	2.89E-02
rs1931565	chr1: 94596867			a / G	0.33	2.00E-01
rs11581939	chr1: 94607234			T / c	0.35	7.70E-02
rs2022378	chr1: 94607607			a / C	0.24	9.07E-01
rs4847286	chr1: 94607848			T / g	0.41	8.22E-02
rs871664	chr1: 94609478			t / C	0.49	3.78E-02
rs2774920	chr1: 94611300			A / g	0.09	5.60E-01
rs12742802	chr1: 94629643			a / C	0.25	5.39E-01
rs1411701	chr1: 94635028	ARHGAP29		a / G	0.38	2.75E-02
rs12044374	chr1: 94635986	ARHGAP29		t / C	0.43	6.87E-02
rs10874840	chr1: 94636836	ARHGAP29		A / g	0.36	1.63E-02
rs12752790	chr1: 94637058	ARHGAP29		T / c	0.43	6.87E-02
rs1048866	chr1: 94638711	ARHGAP29		t / C	0.37	2.91E-02
rs1048854	chr1: 94643531	ARHGAP29	Synonymous_Q891Q	T / c	0.27	5.92E-02
rs11577575	chr1: 94646514	ARHGAP29		a / G	0.23	7.50E-01
rs4847294	chr1: 94657769	ARHGAP29		A / g	0.43	7.99E-02
rs1541098	chr1: 94667970	ARHGAP29		T / c	0.27	9.07E-02
rs2274788	chr1: 94674726	ARHGAP29		T / c	0.24	2.36E-01
rs3789689	chr1: 94685585	ARHGAP29		T / g	0.07	2.75E-01
rs6541343	chr1: 94689027	ARHGAP29		a / G	0.08	1.16E-01
rs12724116	chr1: 94689734	ARHGAP29		A / g	0.15	7.11E-01
rs3789688	chr1: 94691240	ARHGAP29		t / C	0.47	8.34E-04

rs number	Location (bp)[b]	Gene	Exonic variant	Alleles[c]	MAF[d]	P_{trend}-value
rs6673491	chr1: 94693145	ARHGAP29		t / C	0.08	1.18E-01
rs12750249	chr1: 94721660			T / c	0.29	3.39E-03
rs11165101	chr1: 94729088			a / C	0.40	1.71E-04
rs17396055	chr1: 94730954			a / G	0.32	3.47E-03
rs2391472	chr1: 94737583			T / c	0.08	9.28E-02
rs11165110	chr1: 94752469			a / G	0.40	2.19E-04
rs1330855	chr1: 94760885			A / g	0.20	3.21E-02
rs11580391	chr1: 94769368			t / C	0.29	6.70E-03
rs16928	chr1: 94786514			t / C	0.08	1.14E-01
rs17111408	chr1: 94788623			t / C	0.08	1.28E-01
rs12027548	chr1: 94788768			A / g	0.08	1.07E-01
rs11584317	chr1: 94792449			t / C	0.15	1.93E-01
rs2065971	chr1: 94807102			A / c	0.42	2.91E-04
rs2391467	chr1: 94850443			A / g	0.50	2.56E-06
rs1572575	chr1: 94852474			A / g	0.06	1.59E-01
rs12037634	chr1: 94867056			T / g	0.07	3.36E-01
rs11165135	chr1: 94870535			T / g	0.48	1.20E-02
rs6681849	chr1: 94874521			t / G	0.48	1.14E-02
rs10399785	chr1: 94877801			T / c	0.48	1.21E-02
rs4148060	chr1: 94881143			A / g	0.44	1.03E-01
rs10493872	chr1: 94890418	ABCD3		t / G	0.28	6.61E-02
rs12750904	chr1: 94893928	ABCD3		A / g	0.36	1.55E-02

[a]*ARHGAP* + / - 200kb.
[b]NCBI build 37 / hg19.
[c]Lowercase letter denotes the minor allele.
[d]MAF, minor allele frequency based on the entire sample frequencies.
[e]The p_{trend} values below 2.54E-04 (0.05 / 197 SNVs) were interpreted statistically significant.
Significant p-values are highlighted in bold font.

ORIGINAL PAPER

JMS Journal of Medical Science

Short-term effect of intravenous methylprednisolone pulse therapy on glycemic control in patients with normoglycemia and pre-diabetes

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ABSTRACT

Introduction. Systemic steroid therapy leads to disturbances in carbohydrate metabolism. The effect of immunosuppression with intravenous methylprednisolone (IVMP) pulses on glycaemia is not conclusive.
Aim. This study aimed to assess the short-term effect of IVMP therapy in moderate-to-severe Graves' orbitopathy (GO) on glycaemic control in normoglycaemic patients with and without pre-diabetes.
Material and Methods. Twenty-five GO patients treated with IVMP pulses (at initial dose of 6 x 0.5 g once a week, followed by 0.25 g given for 6 consecutive weeksweekly) were recruited and divided into a normoglycaemic group (n = 15, patients without pre-diabetes) and a pre-diabetic group (n = 10, patients with impaired fasting glycaemia (IFG) and/or impaired glucose tolerance (IGT)). Six daily capillary blood glucose measurements were performed at fixed times the day before and on the day of the first pulse administration.
Results. There was a significant increase in the glucose concentration on the day of IVMP administration in both groups of patients compared to the day before drug administration, with 50% of patients showing an increase in blood glucose above 200 mg/dl. There were no statistically significant differences between the two groups.
Conclusions. Methylprednisolone in a high intravenous dose has a tremendous impact on the blood glucose level in normoglycaemic and pre-diabetic patients on the day of drug administration.

Introduction

Therapy with high-dose intravenous glucocorticoids (GCs) is widely and effectively used to treat a variety of inflammatory and autoimmune diseases [1,2]. It is considered as the first-line treatment for moderate-to-severe and active Graves' orbitopathy (GO) by the European Group on Graves' Orbitopathy (EUGOGO) [3]. Intravenously administered GCs are more effective and better tolerated than oral GCs [2-5], however, there is still a risk of serious side effects, e.g. pulmonary embolism, myocardial infarction, severe cerebrovascular events, acute liver damage and sudden death, as well as changes in coagulation status and blood pressure [2,3,5-9]. One of the described side effects is hyperglycaemia, with the influence of GCs on glucose homeostasis being complex. Mechanisms of glucocorticoid-induced diabetes mellitus (DM) include increased insulin resistance, destruction of pancreatic cells, β-cell dysfunction, impaired insulin release, impaired suppres-

Table 1. Summary of studies that investigated glycaemia during intravenous methylprednisolone pulse therapy

Study	Size of the study group	Diagnosis	IVMP regimen	Glycaemic state before IVMP treatment	Glucose-lowering treatment before treatment	Monitoring of glycaemia	Results
Feldman-Billard et al. [12]	224	AON, SU, OIN, CGR, other	250, 500 or 1000 mg of IVMP a day for 3 consecutive days	Patients with and without known DM history Group of patients without DM consisted of normoglycaemic and pre-diabetic patients	All but 1 patient with DM were treated with either oral glucose-lowering agents or insulin	mFBG was measured in all subjects before and after each pulse. In subjects with DM, self-monitoring of capillary blood glucose was performed at least 3 times per day before each meal	Patients without DM showed a 50% increase in mFBG after 1st pulse Diabetic patients showed a 44% increase in mFBG after the 1st pulse
Perez et al. [14]	50	SLE, ITP, MS, AHA, other	1000 mg of IVMP a day for 3 consecutive days	Patients without known DM history	-	mFBG before and after each pulse	68% increase of mFBG after the 1st pulse
Tanaka et. al. [16]	5	GO	500 mg of IVMP - 3 cycles of 3 days a week	Patients without known DM history and normal glucose tolerance	-	Continuous blood glucose monitoring	Glucose levels increased from 4 hours after the administration of IVMP up to midnight, then gradually decreased until morning. The highest glucose level was after dinner, exceeding 200 mg/dl (240–293 mg/dL) in all patients.
Current study	25	GO	Cumulative dose of 4.5 g of IVMP, divided into 12 weekly infusions (6 x 0.5 g, then 6 x 0.25 g)	Patients without DM Divided into two groups: normoglycaemic and pre-diabetic	-	Analysis performed during 1st pulse of IVMP (500 mg) in patients treated with standard 12 pulses of methylprednisolone. Capillary blood glucose measurements 6 times a day – fasting (6:00), two hours after every main meal (11:00, 15:00, 19:00), at 22:00 and 2:00	Increase in capillary blood glucose levels on the day of pulse administration at 19:00, 22:00 and 2:00 in both groups, also at 15:00 in the pre-diabetic group. The highest increase in capillary blood glucose was at 19:00 (mean 204 mg/dL in the non-diabetic group and 203 in the pre-diabetic group). No significant differences between two groups.

AON acute optic neuritis, *SU* severe uveitis, *OIN* ocular infectious diseases, *CGR* corneal graft rejection, *SLE* systemic lupus erythematosus, *ITP* idiopathic thrombocytopenic purpura, *MS* multiple sclerosis, *AHA* autoimmune haemolytic anaemia, *GO* Graves' orbitopathy, *IVMP* intravenous methylprednisolone pulse, *DM* diabetes mellitus, *mFBG* morning fasting blood glucose

sion of hepatic glucose production and inhibited glycogenesis [10,11]. Only a few studies assessed the influence of intravenous methylprednisolone (IVMP) on glucose tolerance (Table 1) [12-16]. Moreover, most studies did not compare patients without diabetes (non-diabetic) to patients with diabetes [12,14]. Some authors suggest that the effect of the IVMP therapy on glucose tolerance in non-diabetic patients is transient and has no clinical relevance, thus these patients do not need any glucose-lowering treatment [12], while others believe that there is evidence that acute hyperglycaemia is a cardiovascular risk factor, independent of the presence of previous diabetes [14,17-19]. Acute hyperglycaemia is associated with an increase in LDL cholesterol oxidation, impaired endothelial function, activation of the coagulation cascade, increased production of pro-inflammatory cytokines and oxidative stress. Therefore, this study aimed to evaluate the short-term influence of IVMP therapy in moderate-to-severe GO on glucose tolerance in patients with normoglycaemia and those with pre-diabetes (impaired fasting glycaemia (IFG) and/or impaired glucose tolerance (IGT)) prior to treatment.

Material and Methods

Patients

The study was conducted at one academic referral centre in the Medical University of Warsaw (WUM). Patients with active, moderate-to-severe GO according to the EUGOGO classification were admitted to the Department of Endocrinology for IVMP therapy from 2012 to 2016. The study included 25 patients: 20 patients with Graves' disease, 4 patients with Hashimoto's thyroiditis and

Table 2. Basic characteristics of patients (n = 25)

	Number of patients (%) or mean ± SD range		
	Normoglycaemic	**Pre-diabetic**	***p*-value**
Number of patients	15 (60%)	10 (40%)	
Impaired fasting glucose	0 (0%)	10 (100%)	
Fasting plasma glucose (normal range – lower than 100 mg/dl)	90 ± 4.92 (81–98)	110 ± 16.17 (100–152)	0.00003
Impaired glucose tolerance	0 (0%)	5 (50%)	
Blood plasma glucose in 2h oral glucose tolerance test (normal range – lower than 140 mg/dl)	97 ± 20.3 (68–133)	134 ± 47.86 (61–192)	0.1
Thyroid disease			
Graves' disease treated for hyperthyroidism	10 (67%)	6 (60%)	1
Graves' disease after radical treatment on levothyroxine	2 (13%)	1 (10%)	1
Euthyroid Graves'	0 (0%)	1 (10%)	0.4
Hashimoto thyroiditis on levothyroxine	3 (20%)	1 (10%)	0.6
Orbitopathy of unknown aetiology	0 (0%)	1 (10%)	0.4
Sex			
Women	9 (60%)	8 (80%)	0.4
Men	6 (40%)	2 (20%)	0.4
Age (years)	50 ± 10 (35–77)	59 ± 10 (43–74)	0.07
Body mass index (kg/m^2)	25 ± 4 (20–34)	26 ± 5 (16–33)	0.7
Current smokers	7 (47%)	2 (20%)	0.2
Past smokers	5 (33%)	3 (30%)	1
Non-smokers	3 (20%)	5 (50%)	0.2
TSH (normal range: 0.27–4.2 µIU/mL)	2.83 ± 1.63 (0.52–6.25)	1.78 ± 1.68 (0.008–5.81)	0.2
fT4 (normal range 12.0–22.0 pmol/L)	16.28 ± 3.6 (12.1–20.9)	18.64 ± 3.73 (12.91–21.96)	0.1
fT3 (normal range: 3.1–6.8 pmol/L)	4.76 ± 0.93 (3.2–6.6)	5.16 ± 0.86 (3.36–6.48)	0.3
Median CAS	4.0	4.5	0.8
Comorbidity			
Hypertension	5 (33%)	4 (40%)	1
Hypercholesterolemia	1 (6%)	1 (10%)	1
Diabetes mellitus	0 (0%)	0 (0%)	
Oral GCs	0 (0%)	0 (0%)	
Hypoglycaemic drugs	0 (0%)	0 (0%)	

1 patient with orbitopathy of unknown aetiology. In total, 16 patients were treated with antithyroid drugs (alone or according to a "block and replace" schedule) and 7 patients received levothyroxine: 3 patients with Graves' disease who were at least 6 months after the last radical treatment (radioiodine therapy or thyroidectomy) and 4 patients with Hashimoto's thyroiditis, 1 patient had euthyroid Graves' disease. The inclusion criteria consisted of (1) active, moderate-to-severe GO; (2) age≥18 years and (3) euthyroidism for at least 1 month. Exclusion criteria were: (1) treatment with oral GCs within the last six months; (2) any other treatment known to significantly alter carbohydrate metabolism (e.g., glucose-lowering drugs) and (3) a clinical diagnosis of diabetes mellitus. Depending on the state of carbohydrate metabolism, patients were divided into two groups: a normoglycaemic group (n = 15, patients without pre-diabetes) and a pre-diabetic group (n = 10, patients with IFG and/or IGT). Diagnosis of pre-diabetes was based on fasting plasma glucose higher than or equal to 100 mg/dl but lower than 126 mg/dl and/ or blood plasma glucose higher or equal to 140 mg/dl but lower than 200 mg/dl in the second hour of oral glucose tolerance test [20]. Clinical characteristics of both groups are shown in Table 2. The study was approved by the Bioethics Committee of the Medical University of Warsaw.

Study design

All patients received IVMP therapy according to the EUGOGO recommendations: starting at a dose of 0.5 g once weekly for 6 weeks, followed by 0.25 g once weekly for 6 weeks (4.5 g cumulative dose). The analysis was performed during the 1st IVMP administration, with an IVMP pulse infusion of 4 hours from 11:00 to 15:00 for all patients. In both groups, six daily capillary blood glucose measurements (glycaemic profile) were performed at fixed times (6:00, 11:00, 15:00, 19:00, 22:00, 2:00) the day before and on the day of the 1st IVMP pulse administration. Patients received a diet consisting of three meals at 9:00, 13:00 and 17:00, with the measurement at 6:00 indicating fasting glucose and measurements at 11:00, 15:00 and 19:00 indicating the glucose level 2 hours after a meal. Capillary blood glucose measurements were analysed using a Glucomaxx glucose meter (Genexo, Warsaw, Poland).

Statistical analysis

All analyses were performed using STATISTICA software ver. 13.3 (StatSoft Polska, Cracow, Poland). Continuous variables are expressed as mean ± standard deviation (SD) or median values. Categorical data were presented as numbers (n) or percentages (%). Comparisons between blood glucose measurements were performed using paired t-tests. Differences between both groups (normoglycaemic and pre-diabetic) were compared using the Mann-Whitney U test. A p-value <0.05 was deemed statistically significant.

Results

Evaluation before intervention

Baseline mean values of six daily capillary blood glucose measurements on the day before the administration of 500 mg IVMP are shown in Table 3. In all patients in the normoglycaemic group, glycaemia remained lower than 200 mg/dl during the day. In one patient (10%) from the pre-diabetic group, glycaemia higher than 200 mg/dl was observed during the day.

Table 3 Changes in glucose concentrations during intravenous methylprednisolone pulse (0.5 g)

	Normoglycaemic group			Pre-diabetic group		
Time of measurement	**Day before**	**Day of the pulse**	***p*-value**	**Day before**	**Day of the pulse**	***p*-value**
6:00	88 ± 6	89 ± 12	0.77	93 ± 10	90 ± 12	0.5
11:00	110 ± 31	106 ± 21	0.6	95 ± 24	102 ± 26	0.6
15:00	114 ± 26	143 ± 34	0.057	100 ± 14	148 ± 28	0.007
19:00	116 ± 20	204 ± 44 (130–318)	0.00003	104 ± 25	203 ± 35 (161–330)	0.001
22:00	101 ± 20	162 ± 37	0.00003	96 ± 20	167 ± 21	0.00002
2:00	93 ± 17	139 ± 25	0.00004	86 ± 11	152 ± 33	0.001

Results are presented as mean ± SD. Statistically significant results are in bold

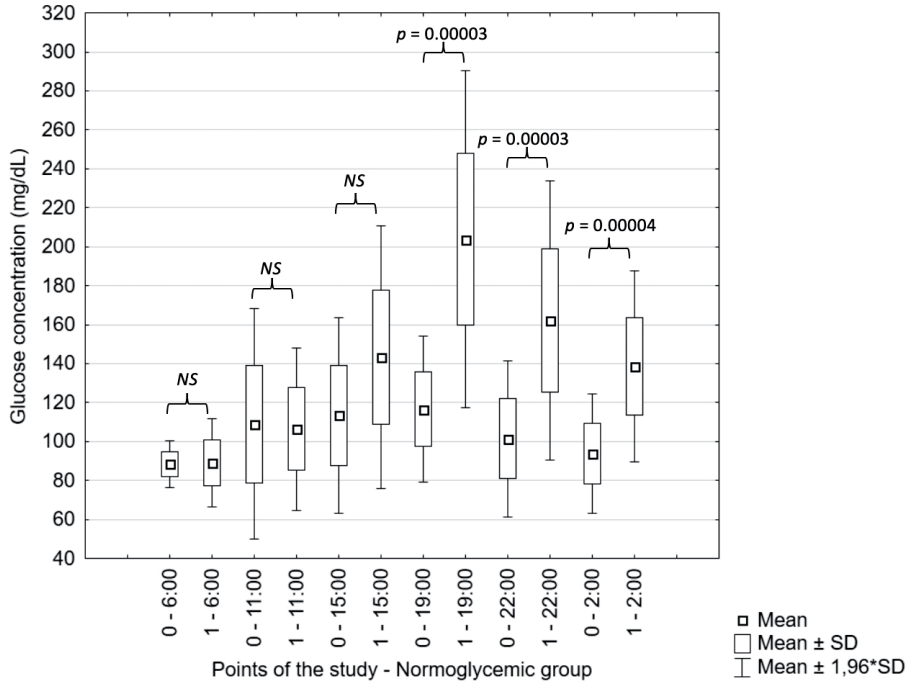

Figure 1. Glucose concentrations during intravenous methylprednisolone pulse (500 mg) in normoglycaemic patients. *NS* non-significant. *0–6:00* day before the pulse, measurement at 6:00, *1–6:00* day of the pulse, measurement at 6:00, *0-11:00* day before the pulse, measurement at 11:00, *1–11:00* day of the pulse, measurement at 11:00, *0-15:00* day before the pulse, measurement at 15:00, *1–15:00* day of the pulse, measurement at 15:00, *0–19:00* day before the pulse, measurement at 19:00, *1–19:00* day of the pulse, measurement at 19:00, *0–22:00* day before the pulse, measurement at 22:00, *1–22:00* day of the pulse, measurement at 22:00, *0–2:00* day before the pulse, measurement at 2:00, *1–2:00* day of the pulse, measurement at 2:00.

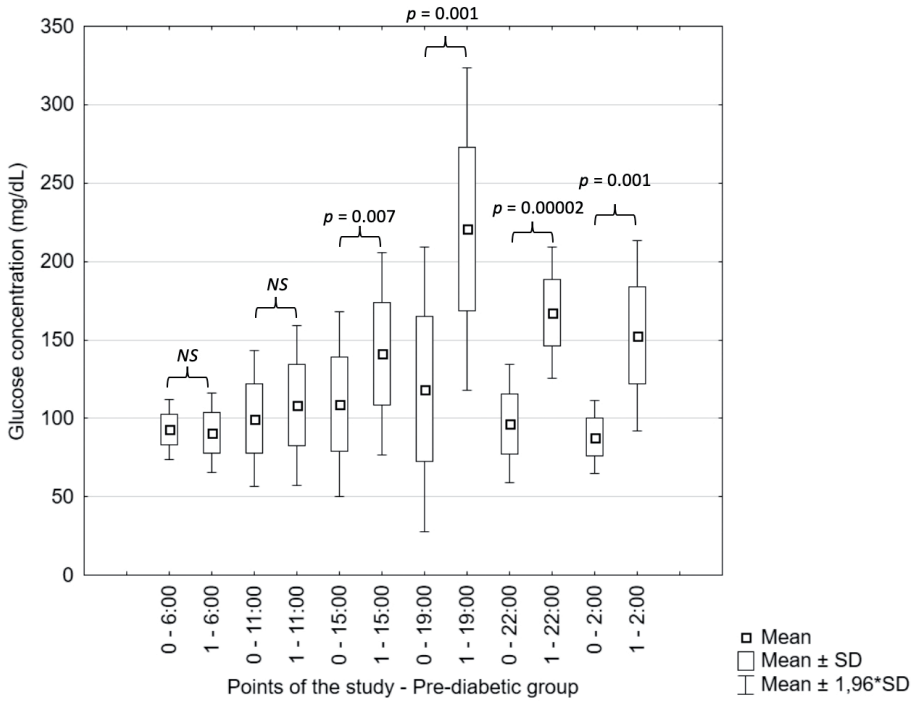

Figure 2. Glucose concentrations during intravenous methylprednisolone pulse (500 mg) in pre-diabetic patients. *NS* non-significant. *0–6:00* day before the pulse, measurement at 6:00, *1–6:00* day of the pulse, measurement at 6:00, *0–11:00* day before the pulse, measurement at 11:00, *1–11:00* day of the pulse, measurement at 11:00, *0–15:00* day before the pulse, measurement at 15:00, *1–15:00* day of the pulse, measurement at 15:00, *0–19:00* day before the pulse, measurement at 19:00, *1–19:00* day of the pulse, measurement at 19:00, *0–22:00* day before the pulse, measurement at 22:00, *1–22:00* day of the pulse, measurement at 22:00, *0–2:00* day before the pulse, measurement at 2:00, *1–2:00* day of the pulse, measurement at 2:00

Short-term influence on single IVMP pulse on glycaemia

Detailed outcomes of capillary blood glucose for the IVMP pulse are shown in Table 3. In the normoglycaemic group, we observed a statistically significant increase in capillary blood glucose levels on the day of pulse administration at 19:00, 22:00 and 2:00 (Figure 1 and Table 3). In the pre-diabetic group, we observed a significant increase in capillary blood glucose levels on the day of pulse administration at 15:00, 19:00, 22:00 and 2:00 (Figure 2 and Table 3). The highest increase in capillary blood glucose compared to the glucose concentration in both groups was observed on the day before the pulse at 19:00, which is 8 hours after the start of the IVMP infusion and 4 hours after its end.

A comparative analysis did not show statistically significant differences between the observed increases in glycaemia between two groups. Detailed outcomes are presented in Table 4 and Figure 3.

Discussion

Hypercortisolism, both endogenous and exogenous, is associated with an increased risk of hyperglycaemia and diabetes mellitus, hence, may occur during the for therapy with oral GCs [21]. However, the influence of therapy with IVMP on glycaemic control is not conclusive, with only a few studies conducted regarding this topic. The results of these studies are summarised in Table 1.

Feldman-Billard et al. [12] and Perez et al. [14] assessed morning fasting glucose one day after IVMP administration, observing a 50% and 68% increase, respectively. Unfortunately, in both studies, the groups of patients were not homogeneous, with patients receiving IVMP due to different indications and varying doses. The studies included patients without diagnosed diabetes mellitus, but they were not divided into two groups (patients with normoglycaemia vs patients with pre-diabetes). Furthermore, both lacked information wheth-

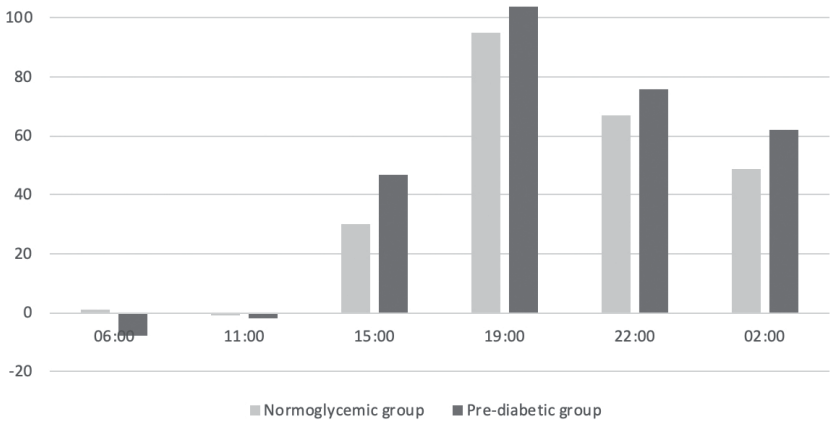

Figure 3. Average increase in glucose concentration at a given measurement point on the day of the intravenous methylprednisolone pulse administration compared to average glucose concentration at the same time of day before

Table 4 Average increase in glucose concentration at a given measurement point on the day of intravenous methylprednisolone pulse (0.5 g) administration compared to average glucose concentration at the same time of day before

	Average increase in glucose concentration		
Time of measurement	Normoglycaemic group	Pre-diabetic group	*p*-value
6:00	1	-7.5	0.2
11:00	-1	-2	0.9
15:00	30	47	0.5
19:00	95	104	0.7
22:00	67	76	0.6
2:00	49	62	0.4

Results are presented as mean. No results were statistically significant

er patients were taking other medications that may affect glycaemia or whether steroids were given at a fixed time. Moreover, in both studies, glycaemia was controlled only in the morning and no measurements were taken during the day.

While Feldman-Billard et al. [12] suggested that the effect of the IVMP therapy on glucose tolerance in patients without DM is transient and has no clinical relevance, thus these patients do not need any monitoring of blood glucose levels or glucose-lowering treatment, Perez et al. [14] stated that there is evidence that acute hyperglycaemia is a cardiovascular risk factor, independently of the presence of previous diabetes, so patients without DM should be monitored and further long-term studies are necessary to identify clinical significance. None of these studies evaluated glucose levels at the time of day when they are most affected, which may explain the controversy.

There is only one study [16] concerning patients without diabetes receiving IVMP pulse therapy in which glucose levels were monitored during the day of the treatment. Unfortunately, the study group was small, consisting of five patients. Regardless, it was noted that glycaemia increased 2–3 hours after IVMP pulse administration, lasting for 12 hours and reaching a peak after dinner, or about 10 hours after administration of the IVMP. Glucose levels exceeded 200 mg/dl after dinner in all patients (ranged from 240 to 293), then gradually decreased until morning.

The present study is unique in that the analysed group of patients was homogenous, with patients receiving IVMP at the same dose due to the same indication. IVMP infusion was administered at the same time and lasted for the same period of time for each patient. Also, patients who received medications that may affect blood glucose levels were excluded. Moreover, six daily capillary blood glucose measurements at fixed times (glycaemic profile) were performed to assess glycaemia at the time that it would be most affected. Furthermore, normoglycaemic and pre-diabetic patients were compared.

In this study, measurements of capillary blood glucose during the day of administration of IVMP pulse in comparison to the day before the drug infusion in both normoglycaemic and pre-diabetic patients showed a significant increase of glucose levels 4 hours after the start of IVMP pulse administration, reaching a peak at about 8 hours. At its peak, the mean levels of glucose were 200 mg/dl in both subsets (ranging from 130 to 318 and 161 to 330 in the normoglycaemic and pre-diabetic groups, respectively). Then, after this glucose peak, glucose levels in subsequent measurements gradually decreased, suggesting that the hyperglycaemic effect of IVMP pulse therapy develops at least 4 hours after the beginning of IVMP pulse administration, achieving a peak 4 hours later, then gradually decreasing. There was no difference between non-diabetic and pre-diabetic patients.

The most important question, however, is whether such a high increase in glucose is of clinical importance, especially considering the whole cycle of IVMP therapy with 12 weekly repeated infusions. Is the administration of glucose-lowering agents required?

The present study has some limitations. First, glucose measurements were derived from capillary blood using a glucose meter and not from serum glucose measurements. Moreover, the study was designed with a relatively small number of patients and included overweight and obese patients, patients did not receive standardised meals, and HOMA-IR was not determined prior to treatment to exclude patients with insulin resistance, all of which may affect glucose levels. Finally, we assessed only the short-term effect of one IVMP pulse administration on glucose levels, not the long-term impact of a whole cycle of IVMP therapy, which consists of 12 pulses. Also, we did not measure HbA1c and glucose tolerance with OGTT after therapy, hence, further research is required to assess the long-term impact.

In conclusion, methylprednisolone in a high intravenous dose has a tremendous impact on blood glucose levels in normoglycaemic and pre-diabetic patients on the day of drug administration.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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

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References

1. Boumpas DT, Chrousos G, Wilder R, Cupps T, Balow J. Glucocorticoid Therapy for Immune-Mediated Diseases: Basic and Clinical Correlates. Annals of Internal Medicine. 1993 Dec 15;119(12):1198-208. https://doi.org/10.7326/0003-4819-119-12-199312150-00007
2. Miśkiewicz P, Kryczka A, Ambroziak U, Rutkowska B, Główczyńska R, Opolski G, Kahaly G, Bednarczuk T. Is high dose intravenous methylprednisolone pulse therapy in patients with Graves' orbitopathy safe?. Endokrynol Pol. 2014;65(6):402-13. https://doi.org/10.5603/EP.2014.0056
3. Bartalena L, Baldeschi L, Boboridis K, Eckstein A, Kahaly GJ, Marcocci C, Perros P, Salvi M, Wiersinga WM. The 2016 European Thyroid Association/European Group on Graves' Orbitopathy Guidelines for the Management of Graves' Orbitopathy. European Thyroid Journal. 2016;5(1):9-26. https://doi.org/10.1159/000443828
4. Stiebel-Kalish H, Robenshtok E, Hasanreisoglu M, Ezrachi D, Shimon I, Leibovici L. Treatment Modalities for Graves' Ophthalmopathy: Systematic Review and Metaanalysis. The Journal of Clinical Endocrinology & Metabolism. 2009 Aug 1;94(8):2708-16. https://doi.org/10.1210/jc.2009-0376
5. Marcocci C, Watt T, Altea MA, Rasmussen AK, Feldt-Rasmussen U, Orgiazzi J, Bartalena L, European Group of Graves' Orbitopathy. Fatal and non-fatal adverse events of glucocorticoid therapy for Graves' orbitopathy: a questionnaire survey among members of the European Thyroid Association. European Journal of Endocrinology. 2012 Feb;166(2):247-253. https://doi.org/10.1530/eje-11-0779
6. Moleti M, Giuffrida G, Sturniolo G, Squadrito G, Campennì A, Morelli S, Puxeddu E, Sisti E, Trimarchi F, Vermiglio F, Marinò M. Acute liver damage following intravenous glucocorticoid treatment for Graves' ophthalmopathy. Endocrine. 2016 Mar 22;54(1):259-268. https://doi.org/10.1007/s12020-016-0928-3
7. Miśkiewicz P, Milczarek-Banach J, Rutkowska-Hinc B, Kondracka A, Bednarczuk T. High-dose intravenous methylprednisolone therapy in patients with Graves' orbitopathy is associated with the increased activity of factor VIII. Journal of Endocrinological Investigation. 2018 Jun 9;42(2):217-225. https://doi.org/10.1007/s40618-018-0907-z
8. Miskiewicz P, Milczarek-Banach J, Bednarczuk T, Opolski G, Glowczynska R. Blood Pressure Profile and N-Terminal-proBNP Dynamics in Response to Intravenous Methylprednisolone Pulse Therapy of Severe Graves' Orbitopathy. International Journal of Molecular Sciences. 2018 Sep 26;19(10):2918. https://doi.org/10.3390/ijms19102918
9. Miśkiewicz P, Jankowska A, Brodzińska K, Milczarek-Banach J, Ambroziak U. Influence of Methylprednisolone Pulse Therapy on Liver Function in Patients with Graves' Orbitopathy. International Journal of Endocrinology. 2018 Oct 21;2018:1-9. https://doi.org/10.1155/2018/1978590
10. Suh S, Park MK. Glucocorticoid-Induced Diabetes Mellitus: An Important but Overlooked Problem. Endocrinology and Metabolism. 2017;32(2):180. https://doi.org/10.3803/enm.2017.32.2.180
11. Clore J, Thurby-Hay L. Glucocorticoid-Induced Hyperglycemia. Endocrine Practice. 2009 Jul;15(5):469-474. https://doi.org/10.4158/ep08331.rar
12. Feldman-Billard S, Lissak B, Benrabah R, Kassaei R, Héron E. Intravenous pulse methylprednisolone therapy in eye disease. Ophthalmology. 2003 Dec;110(12):2369-2371. https://doi.org/10.1016/s0161-6420(03)00818-2
13. Feldman-Billard S, Lissak B, Kassaei R, Benrabah R, Héron E. Short-term tolerance of pulse methylprednisolone therapy in patients with diabetes mellitus. Ophthalmology. 2005 Mar;112(3):511-515. https://doi.org/10.1016/j.ophtha.2004.10.032
14. Perez TH, de Ossio GM, Flores QD, Coria HM, Peña TA, Coria HM, Pérez CG, Peña PS. Glucose disturbances in non-diabetic patients receiving acute treatment with methylprednisolone pulses. Rev Assoc Med Bras (1992). 2012 Jan-Feb;58(1):125-8. Rev Assoc Med Bras (1992). 2012 Jan-Feb;58(1):125-8. PMID 22392327
15. Martinez Martinez M, Abud-Mendoza C, Martínez-Lozano J, Acevedo-Castañeda E, Herrera-van Oostdam D. AB1025 Glycemia and Systemic Blood Pressure in Patients Who Receive Corticosteroid Pulse Therapy. Annals of the Rheumatic Diseases. 2016 Jun;75(Suppl 2):1252.2-1252. https://doi.org/10.1136/annrheumdis-2016-eular.6082
16. Tanaka K, Okada Y, Mori H, Torimoto K, Arao T, Tanaka Y. The Effects of Mitiglinide and Repaglinide on Postprandial Hyperglycemia in Patients Undergoing Methylprednisolone Pulse Therapy. Internal Medicine. 2018;57(1):65-70. https://doi.org/10.2169/internalmedicine.9013-17
17. Loader J, Montero D, Lorenzen C, Watts R, Méziat C, Reboul C, Stewart S, Walther G. Acute Hyperglycemia Impairs Vascular Function in Healthy and Cardiometabolic Diseased Subjects. Arteriosclerosis, Thrombosis, and Vascular Biology. 2015 Sep;35(9):2060-2072. https://doi.org/10.1161/atvbaha.115.305530
18. Yang Z, Laubach VE, French BA, Kron IL. Acute hyperglycemia enhances oxidative stress and exacerbates myocardial infarction by activating nicotinamide adenine dinucleotide phosphate oxidase during reperfusion. The Journal of Thoracic and Cardiovascular Surgery. 2009 Mar;137(3):723-729. https://doi.org/10.1016/j.jtcvs.2008.08.056
19. Lemkes B, Hermanides J, Devries J, Holleman F, Meijers J, Hoekstra J. Hyperglycemia: a prothrombotic factor?. Journal of Thrombosis and Haemostasis. 2010 May 12;8(8):1663-1669. https://doi.org/10.1111/j.1538-7836.2010.03910.x
20. Cosentino F, Grant PJ, Aboyans V, Bailey CJ, Ceriello A, Delgado V, Federici M, Filippatos G, Grobbee DE, Hansen TB, Huikuri HV, Johansson I, Jüni P, Lettino M, Marx N, Mellbin LG, Östgren CJ, Rocca B, Roffi M, Sattar N, Seferović PM, Sousa-Uva M, Valensi P, Wheeler DC, Piepoli MF, Birkeland KI, Adamopoulos S, Ajjan R, Avogaro A, Baigent C, Brodmann M, Bueno H, Ceconi C, Chioncel O, Coats A, Collet J, Collins P, Cosyns B, Di Mario C, Fisher M, Fitzsimons D, Hal-

vorsen S, Hansen D, Hoes A, Holt RIG, Home P, Katus HA, Khunti K, Komajda M, Lambrinou E, Landmesser U, Lewis BS, Linde C, Lorusso R, Mach F, Mueller C, Neumann F, Persson F, Petersen SE, Petronio AS, Richter DJ, Rosano GMC, Rossing P, Rydén L, Shlyakhto E, Simpson IA, Touyz RM, Wijns W, Wilhelm M, Williams B, Aboyans V, Bailey CJ, Ceriello A, Delgado V, Federici M, Filippatos G, Grobbee DE, Hansen TB, Huikuri HV, Johansson I, Jüni P, Lettino M, Marx N, Mellbin LG, Östgren CJ, Rocca B, Roffi M, Sattar N, Seferović PM, Sousa-Uva M, Valensi P, Wheeler DC, Windecker S, Aboyans V, Baigent C, Collet J, Dean V, Delgado V, Fitzsimons D, Gale CP, Grobbee DE, Halvorsen S, Hindricks G, Iung B, Jüni P, Katus HA, Landmesser U, Leclercq C, Lettino M, Lewis BS, Merkely B, Mueller C, Petersen SE, Petronio AS, Richter DJ, Roffi M, Shlyakhto E, Simpson IA, Sousa-Uva M, Touyz RM, Zelveian PH, Scherr D, Jahangirov T, Lazareva I, Shivalkar B, Naser N, Gruev I, Milicic D, Petrou PM, Linhart A, Hildebrandt P, Hasan-Ali H, Marandi T, Lehto S, Mansourati J, Kurashvili R, Siasos G, Lengyel C, Thrainsdottir IS, Aronson D, Di Lenarda A, Raissova A, Ibrahimi P, Abilova S, Trusinskis K, Saade G, Benlamin H, Petrulioniene Z, Banu C, Magri CJ, David L, Boskovic A, Alami M, Liem AH, Bosevski M, Svingen GFT, Janion M, Gavina C, Vinereanu D, Nedogoda S, Mancini T, Ilic MD, Fabryova L, Fras Z, Jiménez-Navarro MF, Norhammar A, Lehmann R, Mourali MS, Ural D, Nesukay E, Chowdhury TA. 2019 ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD. European Heart Journal. 2019 Aug 31;41(2):255-323. https://doi.org/10.1093/eurheartj/ehz486

21. Gulliford M, Charlton J, Latinovic R. Risk of Diabetes Associated With Prescribed Glucocorticoids in a Large Population. Diabetes Care. 2006 Nov 27;29(12):2728-2729. https://doi.org/10.2337/dc06-1499

ORIGINAL PAPER

JMS Journal of Medical Science

Assessment of general movement among infants not at risk of developmental delay

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ABSTRACT

Introduction. The functional assessment of general movements (GMs) is a common test for the developing nervous system. The high predictive validity of abnormal GMs for cerebral palsy has been documented among preterm infants.

Aim. The present study examined whether term infants without any documented risk factors for neurodevelopment delay may benefit from an assessment of GMs.

Methods. One hundred and four infants ranging in age from 1–4 months were evaluated using Prechtl's method, of which, thirty-eight were younger than two months of age and the remaining sixty-eight were older than two months of age (with available detailed neonatal characteristics). The following movements were considered among younger infants, writhing, poor repertoire and cramped synchronised, whereas fidgety, cramped synchronised, poor repertoire, chaotic and abnormal GMs were evaluated in older infants. Infants were classified as 'normal' or 'abnormal' groups based on their presenting GMs. We determined postural positional preference, following Kaplan recommendations, with features categorised as either 'present' or 'absent', as well as activity level and general muscle tone ('normal' or 'abnormal').

Results. Cramped synchronised GMs were observed in seven (18.4%) younger infants and in eleven (16.7%) older infants. There was no difference in the clinical characteristics of children with normal vs. abnormal GMs. Abnormal muscle tone was associated with a higher OR (p = 0.0039) of presenting with abnormal GMs (4.6063; 95%CI: 1.6303–13.0149). Although the infants studied were not at risk for developmental disorders, almost one-fifth required follow-up neurological consultation.

Conclusions. An assessment of GMs should be considered as a universal screening tool among healthy infants without risk factor(s) for developmental deficits.

Introduction

The observation of an infant's movement is an essential part of a child's examination. General Movement (GM) assessment is a reliable tool for identifying infants at risk of neuromotor deficits [1]. GMs are spontaneous motor behaviours with rotations around the limb axes and fluent changes in the direction of movement, generated by central pattern generators (CPGs) located in the brainstem and can be classified as normal and abnormal GMs. Normal GMs consist of "writhing movements", which are present in early foetal life until the end of the second month, and "fidgety movements", which are observable from 3 to 5 months after term. "Writhing" and "fidgety" movements are tiny movements of the neck, trunk, and limbs in all directions and of variable acceleration. Abnormal GMs can include: (a) poor repertoire GMs, which are characterised by the monotonous sequence of movement components; (b) cramped synchronised GMs, which lack the usual smoothness and may be described as rigid limb and trunk contraction; (c) chaotic GMs, which are an abrupt and tremulous movement with large amplitude and high speed; and (d) abnormal fidgety movements with exaggerated amplitude, speed and even jerkiness [1]. If fidgety movements are absent at 3–5 months, the infant may develop severe neurological deficits such as cerebral palsy [2]. At 98% sensitivity, the assessment of GMs is not only a useful clinical instrument for early identification of cerebral palsy but also a good predictor of later cognition and behaviour, even at school age [3]. Normal fidgety GMs have been associated with a high intelligence quotient (IQ) as early as 7–10 years of age [1,4].

The examination of an infant using GMs is safe and non-invasive. During a GM assessment, the child is in the supine position without elicited or intrusive handling. The results of the assessment allow for the application of appropriate therapy to improve motor development as early as possible, which may prevent some motor abnormalities [5].

GM assessment is frequently used as a functional assessment tool for the young nervous system. Indeed, the authors of the Acts of the World Health Organisation recommend performing a functional assessment of children [6–8]. The authors reported that the results of the functional evaluation of children and adolescents correlated with ratings of the children's behaviour, social relations, and school abilities [6–8].

Aim

The study aimed to (1) test whether infants without perinatal risk factors for neurodevelopmental delay should also undergo a GM assessment, and (2) characterise the types of GMs that are present.

Material and Methods

Study group

The study group consisted of 104 infants (57 male, 47 female), 1–4 months of age (mean ± SD: 1.8 ± 0.8 months). All infants from the study group had an appointment in the medical centre because their parents were interested in testing whether their child's motor development was normal. The programme, entitled "Healthy Baby", was free to parents and designed by the Department of Health and Social Affairs. GMs were evaluated in infants by clinicians from the Poznań University of Medical Science. All parents gave written informed consent for their child to complete the assessment. The study was approved by the Bioethical Committee of Poznań University of Medical Sciences, Poland (339/15).

The following inclusion criteria were applied to infants:

1. Patient aged less than 4 months.
2. Patient born in hospitals in the city of Poznań.

The exclusion criteria included:

1. Infants with immediately life-threatening conditions.
2. Active inflammation, infections, or lethal diseases.

Material and Methods

GMs were assessed in infants using the non-invasive method designed by Prechtl. First, the infants were divided into two groups based on age: (1) infants younger than two months of age (n = 38 infants), (2) infants older than two months of age (n = 68 infants). In the younger group, we

tested for writhing, poor repertoire, and cramped synchronised GMs. In the older group, we tested for fidgety, cramped synchronised, poor repertoire, abnormal and chaotic GMs. Infants were further divided into presenting with 'normal' or 'abnormal' GMs.

Postural positional preference was also determined according to recommendations by Sandra L. Kaplan [9,10]. General muscle tone and activity level were assessed according to Prechtl's method and referring to the Neonatal Behavioural Assessment Scale [1,11]. The activity level was also evaluated as a component of GM observation [1]. In all assessments, features were categorised as 'present' or 'absent' for postural positional preference, and as 'normal' or 'abnormal' for activity level and general muscle tone. The parents were also interviewed to assess the neonatal characteristics of their child.

Statistical analysis

The values are expressed as median [interquartile range – IQR] if not stated otherwise. The non-parametric Mann-Whitney U test was used to test for group differences in continuous variables. Two-tailed Fisher's exact test was applied to test for group differences in categorical variables. Data were analysed using STATISTICA 8.1 (StatSoft). All statistical significance levels were set at $p \leq 0.05$.

Results

Infants in this study were born between the 36th and 41st week of gestation, with birth weight ranging from 2,500 to 4,580 g. Umbilical cord blood artery pH ranged from 7.1 to 7.42. Ninety-one of the 104 infants received 10 points in the fifth minute of the Apgar score, and the lowest observed value was 8. The mode of delivery for most patients was natural (n = 52 patients), 40 patients were born by caesarean section, 9 vacuum extraction, and 3 had a forceps delivery. Jaundice was diagnosed in 64 infants and 14 patients required treatment with phototherapy.

Thirty-one out of 38 (81.6%) infants in the younger group (<2 months of age) presented with "writhing" GMs and cramped synchronised GMs were observed in seven (18.4%) infants. None of the infants in the younger group showed poor repertoire GMs. Cramped synchronised GMs were observed in 11 out of the 66 (16.7%) infants in the older age group. No infants in the older group presented with poor repertoire, abnormal or chaotic GMs. To summarise, 17.3% of infants across both groups (younger, older) showed cramped synchronised GMs. Although these infants were not at risk of developmental disorders, almost one-fifth of infants required a follow-up visit to a neurologist.

Infants presenting with normal vs. abnormal GMs did not differ in clinical characteristics, either in the younger (**Table 1**) or older group (**Table 2**). The difference remained non-significant even after combining both groups and considering the entire sample (N = 104).

The distribution of postural preference and general muscle tone did not differ among the younger (**Table 3**) or older infants (**Table 4**). In the younger group, abnormal general muscle tone was more frequent ($p = 0.025$) in infants who presented with abnormal GMs compared to infants presenting with normal GMs. This association did not reach significance in the older group ($p = 0.089$). Consideration of both groups together showed a significant difference ($p = 0.0046$) such that abnormal muscle tone was present in 57.9% (i.e., 11 out of 19) of infants with abnormal GMs vs. 23% (i.e., 20 out of 87) of infants with normal GMs. Abnormal muscle tone was associated with a higher OR ($p = 0.0039$) of presenting with

Table 1. Clinical characteristics of younger infants (<2 months of age) presenting with normal and abnormal GMs

	Normal GMs (n = 31)	Abnormal GMs (n = 7)	*p* value
Apgar score	10 (10–10)	10 (10–10)	0.684
pH	7.32 (7.29–7.36)	7.34 (7.30–7.38)	0.414
Birth weight (g)	3,560 (3100–3980)	3,160 (3010–3950)	0.498
Week of gestation	39 (38–40)	39 (38–41)	0.643

Data presented as median (IQR)

Table 2. Clinical characteristics of older infants (>2 months of age) presenting with normal and abnormal GMs

	Normal GMs (n = 55)	Abnormal GMs (n = 11)	*p* value
Apgar	10 (10–10)	10 (10–10)	0.445
pH	7.32 (7.26–7.38)	7.31 (7.22–7.39)	0.890
Birth weight (g)	3,570 (3160–3840)	3,320 (3230–3720)	0.353
Week of gestation	39 (39–40)	39 (36–40)	0.332

Data presented as median (IQR)

Table 3. Associations between functional parameters and GMs in younger (<2 months of age) infants

		Normal GMs (n = 31)	Abnormal GMs (n = 7)	*p* value
Postural preference	Absent	18	3	0.678
	Present	13	4	
Activity level	Normal	28	5	0.223
	Abnormal	3	2	
General muscle tone	Normal	27	3	0.025
	Abnormal	4	4	

Table 4. Associations between functional parameters and GMs in older (>2 months of age) infants

		Normal GMs (n = 55)	Abnormal GMs (n = 11)	*p*-value
Postural preference	Absent	17	5	0.485
	Present	38	6	
Activity level	Normal	55	11	-
	Abnormal	0	0	
General muscle tone	Normal	39	5	0.159
	Abnormal	16	6	

abnormal GMs (4.6063; 95% CI [confidence interval]: 1.6303–13.0149). There were just five infants in the younger group who presented with abnormal activity levels. Two of the five infants with abnormal activity levels also showed abnormal GMs. No infants in the older group presented with abnormal activity levels (**Table 4**).

To summarise, although infants were not at risk of developmental disorders, 18% of infants across both groups required a follow-up visit to a neurologist.

Discussion

The present study demonstrated that almost one in five infants presented abnormal GMs. We assessed healthy full-term infants without risk factors for developmental delays. Although the predictive validity of abnormal GMs for cerebral palsy is better in infants born preterm, we demonstrated that GMs should also be considered in infants born at term [2,12]. GMs in healthy infants is a useful clinical instrument for the early identification of not only cerebral palsy, but also a good predictor of later cognition, attention, and behavioural problems at school age [3,13]. The observation of movement should be a routine assessment within the first few months of life in all children [13,15,16], which is in line with World Health Organisation recommendations (b761, b7610, International Classification of Functioning, Disability and Health: Children and Youth Version) [7]. Early identification of disordered movement may be a marker of early brain impairment and/or dysfunction. Disordered GMs may have more predictive utility in preterm infants compared to term infants because brain lesions are more heterogeneous in full-term infants. Importantly, GM assessments have a sensitivity and specificity of 98% and 95%, respectively. Furthermore, compared to magnetic resonance imaging, brain ultrasound, and traditional neurological examinations, GM assessments are quick, non-invasive, and cost-effective [14].

The present study indicated that infants, particularly in the younger group and presenting with abnormal GMs, frequently showed abnormal muscle tone. Physiological hypertonia of term infants in the first two months of life should not always be a cause for concern for clinicians because hypertonia may be an expression of increased motoneuronal excitability which subsequently decreases around 3 months of age [17]. Nonetheless, abnormal muscle tone may be a symptom of hypoxic-ischaemic encephalopathy with additional characteristics of perinatal features such as Apgar < 5 in the 5th minute and pH ≤ 7 [18]. Importantly, infants in the present study did not have such risk factors. Our results suggest that general muscle tone may be an important feature that should be assessed in all infants [3,19,20] to evaluate motor development [21,22]. We and others also recommend examining muscle tone not only with the "pull to sit" manoeuvre but also in several positions (e.g., supine, horizontal, vertical, and prone) [21]. Abnormal muscle tone may also be correlated with autism spectrum disorder [23].

We demonstrated that, even in a group of no-risk infants, a subset may require a follow-up examination by a neurologist. The application of a GM assessment should allow physicians or therapists to determine whether an infant needs additional examination or therapy with good predictability [3].

Limitations of the study include a relatively small group of healthy infants and a limited number of physiological variables studied. Most infants included in the present study were eutrophic, born at term, and with proper birth weight. Moreover, we assessed infants who lacked significant developmental risk factors, such as intraventricular haemorrhage, hypoxia, acidosis, Apgar score < 7, extremely low birth weight, or extremely early week of gestation. We also did not evaluate individual infant developmental trajectories.

Strengths of this study should also be noted. In contrast to previous studies, the present study assessed GMs among a group of healthy infants. Parents who were interested in whether their child showed appropriate motor development also confirmed that their child's development appeared normal. Nonetheless, the assessment of GMs may help to identify infants who should visit specialists, such as a neurologist.

Conclusion

In conclusion, even in a group of infants who were not at risk for cerebral palsy, a subset of infants required follow-up consultation, thus, GMs should be assessed in all infants. Early assessment provides the opportunity to help infants as early as possible, which has positive effects on long-term development.

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

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References

1. Nakajima Y, Einspieler C, Marschik PB, Bos AF, Prechtl HF. Does a detailed assessment of poor repertoire general movements help to identify those infants who will develop normally?. Early Human Development. 2006 Jan;82(1):53-59. https://doi.org/10.1016/j.earlhumdev.2005.07.010
2. Einspieler C, Prechtl HFR. Prechtl's assessment of general movements: A diagnostic tool for the functional assessment of the young nervous system. Mental Retardation and Developmental Disabilities Research Reviews. 2005;11(1):61-67. https://doi.org/10.1002/mrdd.20051
3. Ferrari F, Cioni G, Einspieler C, Roversi MF, Bos AF, Paolicelli PB, Ranzi A, Prechtl HFR. Cramped Synchronized General Movements in Preterm Infants as an Early Marker for Cerebral Palsy. Archives of Pediatrics & Adolescent Medicine. 2002 May 1;156(5):460. https://doi.org/10.1001/archpedi.156.5.460
4. Hadders-Algra M. General movements: a window for early identification of children at high risk for developmental disorders. The Journal of Pediatrics. 2004 Aug;145(2):S12-S18. https://doi.org/10.1016/j.jpeds.2004.05.017
5. Smith T, Groen AD, Wynn JW. Randomized Trial of Intensive Early Intervention for Children With Pervasive Developmental Disorder. American Journal on Mental Retardation. 2000;105(4):269. https://doi.org/10.1352/0895-8017-(2000)105<0269:rtoiei>2.0.co;2
6. Stellwagen L, Hubbard E, Chambers C, Jones KL. Torticollis, facial asymmetry and plagiocephaly in normal newborns. Archives of Disease in Childhood. 2008 Apr 1;93(10):827-831. https://doi.org/10.1136/adc.2007.124123

7. van Vlimmeren LA, van der Graaf Y, Boere-Boonekamp MM, L'Hoir MP, Helders PJM, Engelbert RHH. Effect of Pediatric Physical Therapy on Deformational Plagiocephaly in Children With Positional Preference. Archives of Pediatrics & Adolescent Medicine. 2008 Aug 1;162(8):712. https://doi.org/10.1001/archpedi.162.8.712
8. Brazelton TB, Nugent JK. The Neonatal Behavioral Assessment Scale. London: Mac Keith Press; 2011.
9. Einspieler C, Bos AF, Libertus ME, Marschik PB. The General Movement Assessment Helps Us to Identify Preterm Infants at Risk for Cognitive Dysfunction. Frontiers in Psychology. 2016 Mar 22;7. https://doi.org/10.3389/fpsyg.2016.00406
10. Spittle A, Orton J, Anderson PJ, Boyd R, Doyle LW. Early developmental intervention programmes provided post hospital discharge to prevent motor and cognitive impairment in preterm infants. Cochrane Database of Systematic Reviews. 2015 Nov 24;. https://doi.org/10.1002/14651858.cd005495.pub4
11. Gajewska E, Sobieska M, Kaczmarek E, Suwalska A, Steinborn B. Achieving Motor Development Milestones at the Age of Three Months May Determine, but Does Not Guarantee, Proper Further Development. The Scientific World Journal. 2013;2013:1-11. https://doi.org/10.1155/2013/354218
12. Hodges K, Wong MM. Psychometric characteristics of a multidimensional measure to assess impairment: The Child and Adolescent Functional Assessment Scale. Journal of Child and Family Studies. 1996 Dec;5(4):445-467. https://doi.org/10.1007/bf02233865
13. International Classification of Functioning, Disability and Health (ICF). https://www.who.int/classifications/icf/en
14. Gower L, Jenkins D, Fraser JL, Ramakrishnan V, Coker-Bolt P. Early developmental assessment with a short screening test, the STEP, predicts one-year outcomes. Journal of Perinatology. 2018 Oct 9;39(2):184-192. https://doi.org/10.1038/s41372-018-0234-4
15. Chugani HT, Phelps ME, Mazziotta JC. Positron emission tomography study of human brain functional development. Annals of Neurology. 1987 Oct;22(4):487-497. https://doi.org/10.1002/ana.410220408
16. International classification of functioning, disability and health: children and youth version: ICF-CY. https://apps.who.int/iris/handle/10665/43737
17. Fjørtoft T, Grunewaldt KH, Løhaugen GCC, Mørkved S, Skranes J, Evensen KAI. Assessment of motor behaviour in high-risk-infants at 3months predicts motor and cognitive outcomes in 10years old children. Early Human Development. 2013 Oct;89(10):787-793. https://doi.org/10.1016/j.earlhumdev.2013.06.007
18. Prechtl HF, Einspieler C, Cioni G, Bos AF, Ferrari F, Sontheimer D. An early marker for neurological deficits after perinatal brain lesions. The Lancet. 1997 May;349(9062):1361-1363. https://doi.org/10.1016/s0140-6736(96)10182-3
19. Einspieler C, Marschik PB, Milioti S, Nakajima Y, Bos AF, Prechtl HF. Are abnormal fidgety movements an early marker for complex minor neurological dysfunction at puberty?. Early Human Development. 2007 Aug;83(8):521-525. https://doi.org/10.1016/j.earlhumdev.2006.10.001
20. Bruggink JL, Einspieler C, Butcher PR, Van Braeckel KN, Prechtl HF, Bos AF. The Quality of the Early Motor Repertoire in Preterm Infants Predicts Minor Neurologic Dysfunction at School Age. The Journal of Pediatrics. 2008 Jul;153(1):32-39.e1. https://doi.org/10.1016/j.jpeds.2007.12.047
21. Yuge M, Marschik PB, Nakajima Y, Yamori Y, Kanda T, Hirota H, Yoshida N, Einspieler C. Movements and postures of infants aged 3 to 5months: To what extent is their optimality related to perinatal events and to the neurological outcome?. Early Human Development. 2011 Mar;87(3):231-237. https://doi.org/10.1016/j.earlhumdev.2010.12.046
22. Papazian O. Encefalopatía hipóxica-isquémica neonatal [Neonatal hypoxic-ischemic encephalopathy]. Medicina (B Aires). 2018;78(Suppl. 2):36-41. PMID 30199363
23. Razaz N, Cnattingius S, Persson M, Tedroff K, Lisonkova S, Joseph KS. One-minute and five-minute Apgar scores and child developmental health at 5 years of age: a population-based cohort study in British Columbia, Canada. BMJ Open. 2019 May;9(5):e027655. https://doi.org/10.1136/bmjopen-2018-027655

REVIEW PAPER

JMS Journal of Medical Science

Asymmetric properties of heart rate microstructure

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ABSTRACT

The duration of each cardiac cycle is measured on an ECG as the distance between the peaks of consecutive R waves (RR interval), with the inverse value corresponding to the heart rate (HR) changing in a beat-to-beat manner. HR accelerations are reflected as shortenings of the RR intervals, HR decelerations as the lengthening of RR intervals. HR asymmetry is a physiological phenomenon caused by an unequal input of HR decelerations and accelerations to the HR variability. Naturally occurring consecutive values of RR intervals create a time series composed of acceleration and deceleration runs of differing lengths. For example, a single HR acceleration, a pair of HR decelerations, a run consisting of five consecutive HR decelerations or a run composed of eight accelerations in a row. These runs make up the so-called heart rate microstructure that has asymmetric properties due to the unequal contribution of acceleration and deceleration runs. The asymmetry of the HR microstructure is physiological in healthy individuals, however, the asymmetric properties can be significantly altered in some clinical conditions, such as myocardial infarction, obstructive sleep apnoea, chronic obturatory pulmonary disease or sepsis in infants. An abnormal HR microstructure has predictive value in survivors of myocardial infarction or patients with clinical indications for exercise treadmill stress test, e.g., for total mortality. In this review, we present and explain how the asymmetric properties of HR microstructure can be quantified, summarising the available data regarding the clinical and predictive value of this phenomenon and its analysis.

The sinus node is the only physiological pacemaker of the human heart and its electrical depolarisations are the major but not the sole determinant of the length of each cardiac cycle [1–11]. In a normal ECG, the direct activity of the sinus node is practically invisible, but its indirect effects on the atria are observed as the P wave (**Figure 1**). After the right and left atria are depolarised, the electrical potential is conducted through the atrial node, His Bundle system and the Purkinje fibres finally reaching the myocardial cells of both ventricles. Each QRS complex in ECG proves that the right and left ventricles have been depolarised [1,3,7,11]. The duration of the cardiac cycle can be measured as the distance between either two consecutive P waves or two successive QRS complexes (usually R waves). For practical reasons, as the peak of the R wave is sharp

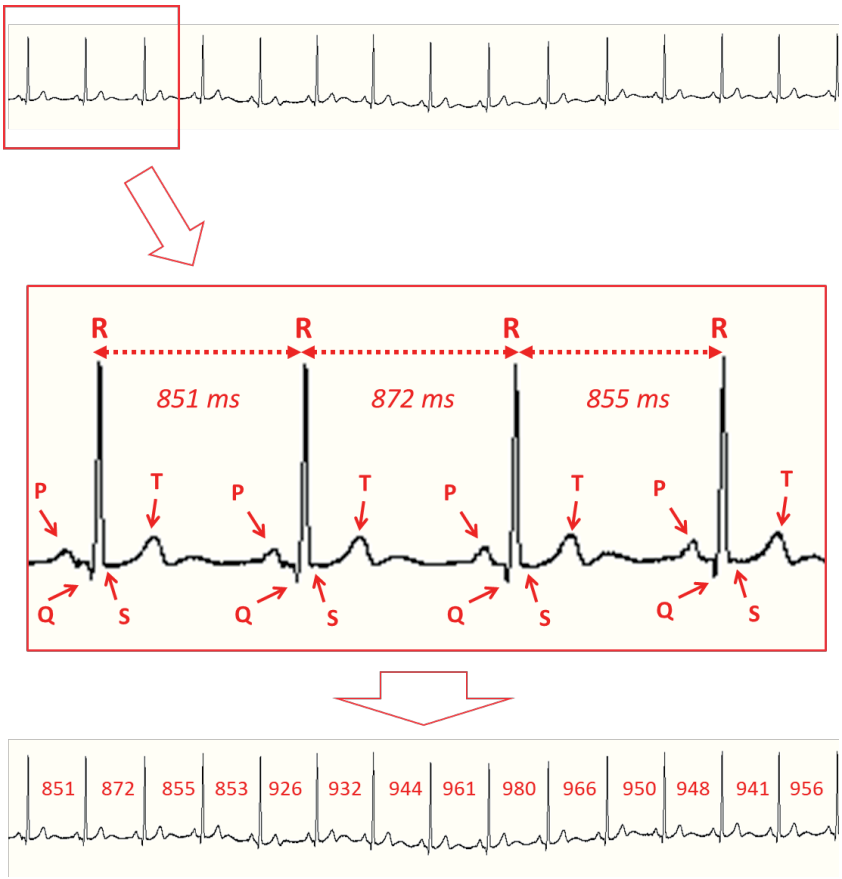


Figure 1. The upper panel shows a strip of normal ECG. The middle panel is zoomed at the first three P-QRS-T waves with the presentation of the distances between the consecutive QRS complexes or peaks of R waves. The measured distances are expressed in milliseconds (ms). The bottom panel shows the same ECG strip with measured RR intervals

and better defined than the front or maximum of a P wave, it is more accurate to measure RR, not PP intervals. Therefore, RR intervals, i.e., the distances between consecutive R waves, are the commonly accepted measure of the duration of cardiac cycles [1,3].

Momentary heart rate (HR) is a simple mathematical inverse of the duration of the cardiac cycle or RR interval. Longer RR intervals correspond to a slower HR, shorter RR intervals to faster HR, with the length of RR intervals or HR changing with each beat. Compared with the previous cardiac cycle, the next cycle can shorten or lengthen, evident as the HR acceleration or deceleration, respectively.

The sinus node is a complex structure composed of many cells, each of which can initiate action potential depolarising other cells within this node, as well as the remaining cardiomyocytes of the whole heart [1,2,4–11]. There are many variations in the sinus node shape and size but generally, it is a crescent-like or spindle-shaped structure consisting of head, body and tail, with a maximal length of 14 mm, height up to 8 mm and width up to 4 mm [1,5]. One to ten radiations of different length and orientation extend from it [5]. Typically, the spindle- and spider-shaped cells, which are smaller, less striated and paler staining, have electrophysiologic features characteristic of pacemaker cells, including spontaneous beating under physiologic conditions. The remaining cells of different histological structure serve to transmit the electrical depolarisation to other cells or as working myocytes and tissue support [1,2,8,9].

Usually, the action potential from the sinus node has a unifocal origin, i.e. a single cell or a group of tightly localised pacemaking cells initiates the depolarisation wave. However, frequently, the action potential comes from multiple pacemaking cells spaced at different parts of the sinus node, which generate multiple wavefronts

coalescing into one depolarisation wave in 10 to 15 ms. Sometimes, a couple of pacing cells from 2–5 foci located more than 1 cm apart depolarise asynchronously with short lags 1 to 5 ms between the activation waves. Regardless of the type, all forms of activation are transferred through discrete sinoatrial exit pathways to the right atrium and the remaining parts of the heart, that is, there is no single leading pacemaker cell, with various types of physiological initiation of the sinus rhythm possible in a healthy human [1,2,7]. The initiation and end of the action potential are complicated, depending on several physiological intracardiac and extracardiac factors, for example, the autonomic innervation, oxygen supply, metabolites and hormones [1,3,4,11]. Taking together all these processes, the probability that different cells within the sinus node will generate an action potential of precisely the same duration in two consecutive cardiac cycles is extremely low.

Beat-to-beat change in the duration of RR intervals of sinus origin is a normal physiological phenomenon [3,12,13]. Its typical example is respiratory sinus arrhythmia: HR accelerates during inspiration and decelerates during expiration [14,15]. Many other mechanisms than breathing contribute to such instant changes of RR intervals, with the whole phenomenon known as heart rate variability (HRV) [3,12, 13,15]. The physiology and clinical value of HRV have been extensively studied for over four to five decades and it has earned dedicated guidelines [12,13]. In 2006, our group published the first paper on the existence of another genuine physiological phenomenon related to HRV – heart rate asymmetry (HRA) [16]. Based on studies in healthy individuals, we demonstrated that HRA is a phenomenon caused by the different behaviour of HR accelerations and decelerations, which unequally contribute to the short-, long-term and total HRV as well as the complexity and microstructure of HR [16–24]. **Figure 2** presents a local tachogram with consecutive RR intervals with the Poincare plot (one of the methods for HRV analysis) of RR intervals

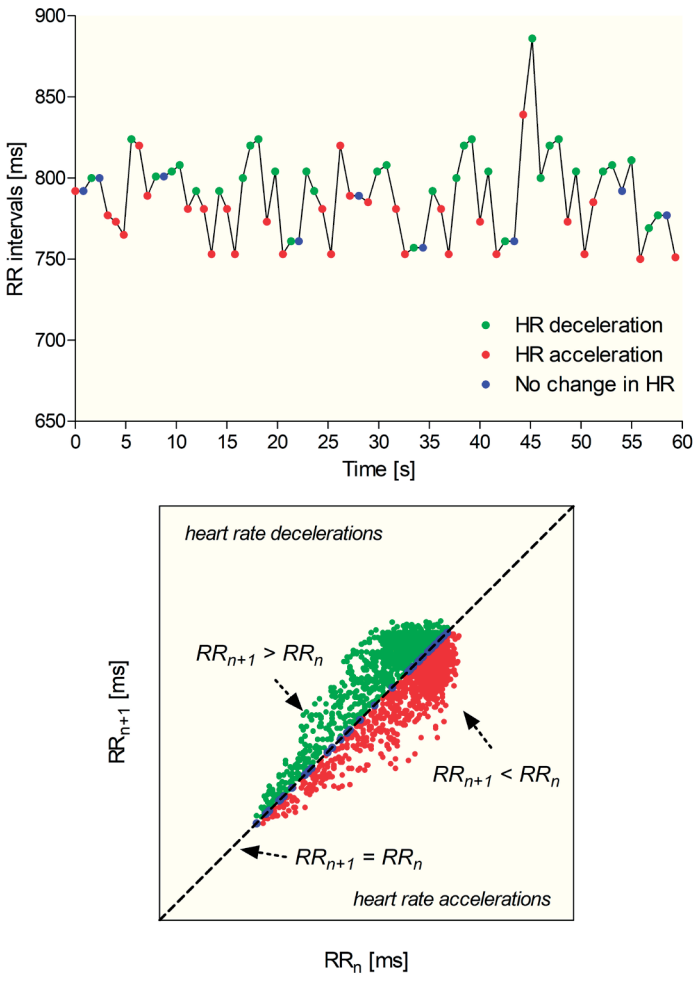


Figure 2. Upper panel shows a local tachogram of consecutive RR intervals from ECG recording of 1-minute duration. RR intervals longer than the previous one are labelled as HR deceleration (green dots), shorter as HR acceleration (red dots), or of the same duration, as no change HR (blue dots). The lower panel shows the Poincare plot of all RR intervals in the space of (RR_{n+1}, RR_n) corresponding to all pairs of two consecutive beats (RR_n and RR_{n+1}). This plot depicts the distribution and relation between such pairs, clearly separating HR decelerations (green dots) from accelerations (red dots). Of note, points described by pairs of RR intervals with precisely the same duration ($RR_n = RR_{n+1}$) are placed on the identity line (broken diagonal line), represented by blue dots in Figure 2. The Poincare plot of RR intervals in the space (RR_n, RR_{n+1}) explicitly visualises the existence of HRA

showing the clear separation between HR accelerations and decelerations.

In this review, the concept of the HR microstructure and its asymmetric properties [20,25] will be explained in detail. Additionally, we summarise the clinical findings and show how sampling frequency of ECG can substantially change the number of acceleration, deceleration and neutral runs making up the HR microstructure.

Heart rate microstructure

Consecutive values of HR, e.g., 75, 76, 74, 72, 78, 80 beats/minute, or RR intervals like 800, 789, 811, 833, 769, 750 ms, create sequences or a series of data points ordered according to their appearance in time, known as the HR time series or RR interval time series, respectively. An RR interval time series is the primary data source for computations of both HRV and HRA. This review will focus only on the RR interval time series.

The order of RR intervals in an ECG of a healthy individual is not random, rather the net result of spontaneous depolarisations of the sinus node modulated by many physiological mechanisms [1,3,12,13]. **Figure 3** shows two different tachograms of two-minute duration composed from precisely the same RR interval data. In the first case, the order of RR intervals is physiological and comes from an ECG acquired during slow-paced breathing at a rate of 6 breaths/minute, whereas the original order of RR intervals from the same ECG has been destroyed by random shuffling in the second case. The mean value and standard deviation of all RR intervals for both data sets are precisely the same, i.e. 1068 ms and 115.2 ms, respectively, however, as clearly visible, the microstructure of both RR time series is entirely different. All RR intervals in the dataset with the original order of RR interval follow breathing (12 waves correspond to 12 breathing cycles recorded during 2 minutes), for the shuffled RR intervals there is no clear and repeated pattern.

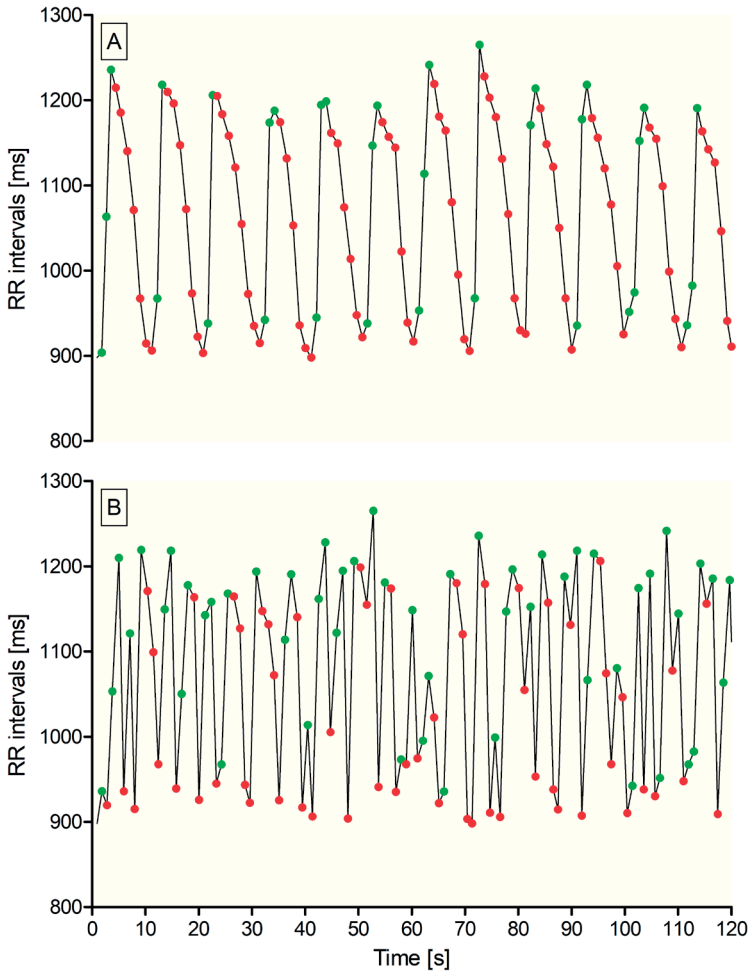


Figure 3. Panel A shows oscillations of RR intervals which are in concordance with the paced breathing at a rate of 6 breaths/minute. This tachogram starts with HR decelerations (green dots) corresponding to expiration, followed by HR accelerations during inspiration, this pattern repeats during each breathing cycle. Panel B shows the same RR intervals but with completely disrupted physiological order, i.e. all RR intervals are randomly shuffled. The distribution of HR accelerations and decelerations is random, with no repeated pattern

As evident in **Figure 3**, for the slow breathing dataset, all RR intervals cluster in groups of either decelerations or accelerations, the so-called monotonic runs. In such a run, consecutive values always change in one direction, either increasing or decreasing. For RR intervals, the monotonic run means that cardiac cycles either prolong for HR decelerations or shorten for HR accelerations. In **Figure 3**, there are runs (or series) of RR intervals of different length, forming two to four consecutive HR decelerations and six to eight successive HR accelerations for the physiological data. In contrast, for RR intervals with the shuffled order, these data are more scattered, with the longest monotonic runs much shorter for both HR accelerations and decelerations. Of note, single HR decelerations and accelerations become more common.

For the analysis of the HR microstructure, all RR intervals are separated into monotonic runs of different length as shown in **Table 1** [24–26]. The shortest possible change in RR interval is a single deceleration or acceleration, such singlets are also termed a run, i.e. a deceleration run of length 1 (DR1) or an acceleration run of length 1 (AR1). Two consecutive RR intervals which either increase or decrease their duration are pairs of decelerations (DR2) and accelerations (AR2). Runs of much longer length are naturally occurring, for instance of the length of ten consecutive decelerations (DR10) or twelve accelerations in a row (AR12).

Simple counting statistics are applied to quantify the number of HR acceleration and deceleration runs, the total number of beats in the runs of specific length is counted, then divided by all normal RR intervals [24–26]. The relative contribution of each type of monotonic run to all sinus beats is presented, usually as a percentage or sometimes as a part of 1 [24–26]. For instance, 15 accelerations forming five runs of three consecutive accelerations (AR3) in a recording of 300 sinus RR intervals have a relative contribution of 5%. In another example, in a longer recording composed of 100,000 RR intervals of sinus origin, 6,567 single decelerations (DR1) have a contribution of 6.57%, 2,332 RR intervals in the form of deceleration pairs (DR2) are quantified as 2.33%, 492 RR intervals forming triples of decelerations (DR3) correspond to 0.49% contribution whereas

RR interval [ms]	Type of HR change	Number of RR intervals changing in the same direction	The label of monotonic run
898	reference value	-	
904	deceleration	2	DR2
954	deceleration		
940	acceleration	1	AR1
948	deceleration	1	DR1
930	acceleration	3	AR3
918	acceleration		
902	acceleration		
927	deceleration	6	DR6
933	deceleration		
945	deceleration		
953	deceleration		
960	deceleration		
968	deceleration		
960	acceleration	2	AR2
942	acceleration		
950	deceleration	1	DR1
948	acceleration	4	AR4
938	acceleration		
931	acceleration		
925	acceleration		
936	deceleration	1	DR1
920	acceleration	1	AR1
927	deceleration	2	DR2
941	deceleration		

Table 1. An example of proper identification of each RR interval as HR deceleration or acceleration. The identification process is simple and based on the comparison of duration of each specific RR interval with the previous RR interval. The number of RR intervals changing in the same direction (either prolonging for HR decelerations or shortening for accelerations) is counted, with specific monotonic runs labelled based on the number of RR intervals changing in the same direction

84 RR intervals found as runs of four decelerations contribute to 0.08% of all sinus beats. The same approach of counting statistics is also applied for reporting the contribution of premature ventricular (or supraventricular) beats to all recorded beats in the longer Holter ECG recordings, for example of 24-hour duration. In such reports, the relative number of ventricular beats is shown, for example as 0.5% or 19%.

In 2011, we showed that the HR microstructure had asymmetric properties in our physiological study of eighty-seven ECG recordings of up to 24-hour duration acquired from healthy people [24]. The number of acceleration runs was usually significantly higher (with the exceptions of AR3s vs DR3s and AR4s vs DR4s) than deceleration runs. Additionally, the longest runs in the same people came from 24 accelerations (AR24) and only 19 decelerations (DR19), with the average longest runs formed of over 15 accelerations and over 12 for decelerations.

Table 2 [25] summarises the relative contributions of RR intervals creating acceleration and deceleration runs to the total number of sinus beats in the 24-hour ECG recordings from healthy people. It is visible that, except for monotonic runs of the length 3 and 4, in the majority of other lengths, acceleration runs have a significantly larger contribution to all sinus beats in these recordings. For monotonic runs of the length 3, there were more DRs than ARs, while the number of monotonic runs of the length 4 was comparable both for decelerations and accelerations.

To check whether the asymmetric properties of the monotonic runs method were not artificially generated, we compared the distribution of the acceleration and deceleration runs after shuffling the original order of RR intervals into a random one in each of the 87 recordings [24]. For the shuffled data, there were no significant differences between acceleration and deceleration runs of any length. Additionally, the longest monotonic runs were composed of nine consecutive beats (AR9 and DR9), and the averaged longest runs were length seven both for decelerations and accelerations (AR7 and DR7). Interestingly, there were significantly fewer runs which were shorter and more runs which were longer for RR interval time series with the original order than after its disruption by random shuffling. Thus, we concluded that the input of HR accelerations and decelerations to the distribution of monotonic runs is unequal, hence asymmetric, that the distribution of acceleration runs and deceleration runs differs between physiological and shuffled data, with some not completely understood physiological mechanisms creating longer monotonic runs at the cost of shorter runs (i.e., singlets and pairs of HR accelerations and decelerations) [24].

Also, in 2011, we published, in a co-operation with the group of Professor Georg Schmidt from Munich in Germany, the first clinical study on the HR microstructure [26]. We analysed the predictive value of HR deceleration runs measured in the 24-hour ECGs from 1455 (training sample) and 946 (validation sample) survivors of myocardial infarction. Patients at high risk of death in the long-term follow-up of median duration of 24 months had significantly more single decelerations (DR1) and fewer deceleration runs of the length between 2 and 10 (DR2-DR10) than the low-risk individuals. The reduced number of

Runs of the length	Deceleration runs [%]		Acceleration runs [%]		P-value
	Median	IQR	Median	IQR	
1	12.64	5.60	13.18	5.03	0.003
2	13.71	4.89	14.63	3.54	<0.001
3	8.71	4.16	7.16	2.59	<0.001
4	3.54	1.98	3.55	1.33	n.s.
5	1.82	1.28	2.32	1.33	<0.001
6	1.01	0.94	1.60	1.29	<0.001
7	0.49	0.53	0.95	0.91	<0.001
8	0.23	0.25	0.56	0.61	<0.001
9	0.1	0.12	0.32	0.38	<0.001
10	0.04	0.06	0.16	0.21	<0.001
11	0.02	0.04	0.08	0.1	<0.001
12	0.01	0.02	0.05	0.08	<0.001

Table 2. Comparison of the relative contributions of acceleration runs and deceleration runs of different length to the total number of sinus beats in the 24-hour ECG recordings from healthy people. The statistical comparison was made with the nonparametric Mann-Whitney test. (IQR – interquartile range) [25]

DR4 up to 0.05% was the best single predictor of three separate modes of premature death, i.e., all-cause mortality, cardiac death, and sudden cardiac death both in the training and validations samples, whereas patients with a preserved number of DR2 >5.4%, DR4 >0.05%, and DR8 >0.005% belonged to the low-risk group of all observed death-related clinical outcomes.

These relative values require some additional comment. If in a 24-hour ECG of a myocardial infarction survivor there are 100,000 sinus beats of which more than 5,400 HR decelerations are in DR2s, more than 50 in DR4s and more than five in DR8, then such a person is at low risk of premature death, whereas if there are 50 or fewer RR intervals which form DR4, then the patient is at an increased risk. Although not presented in that paper in the form of numbers, similar findings were made for HR acceleration runs and commented on in the discussion. The reduced relative numbers of AR2-AR10 were found in high-risk patients, although the prognostic value of acceleration runs was lower in comparison to the deceleration runs.

In general, the distribution of deceleration and acceleration runs in survivors of myocardial infarction who were at an increased risk of premature death converged to the distribution of the shuffled RR intervals as in our physiological study with 24-hour ECGs from healthy individuals. In other words, mechanisms responsible for forming HR accelerations and decelerations into longer runs were preserved in low-risk and attenuated in high-risk post-infarction patients.

We also tested, in co-operation with Professor Tuomo Nieminen from Oulu in Finland, the predictive value of single accelerations and decelerations in pre-exercise ECGs of at least 1-minute duration collected in 944 consecutive patients from the Finnish Cardiovascular Study [27]. All patients had clinical indications for the treadmill exercise test, after which they were followed for a mean of nearly 57 months. Those with increased relative numbers of AR1 >16.85% or DR1 >17.7% had a significantly higher total mortality than other patients. Increased numbers of AR1 and DR1 were also associated with a higher risk for cardiovascular death and sudden cardiac death. As the mean duration of pre-exercise ECGs was short, between 1 minute and a couple of minutes, studying the predictive value of longer runs was impossible for statistical reasons (too low statistical power). However, there was an essential similarity to the study in post-infarction patients – high-risk patients in these two different groups had an increased number of AR1s and DR1s. These findings again highlight that in the high-risk cardiac patients, the numbers of AR1 and DR1 increased as in the case of the shuffled data from healthy people.

Billois et al. compared the asymmetric properties of HR microstructure in 32 premature infants, 16 of whom were infected and at risk of sepsis [28]. The study was performed with the use of 30-minute ECGs, and the authors analysed the absolute numbers of HR decelerations. Significant differences were observed between infants with and without infection in the number of deceleration but not acceleration runs. Infants at an increased risk of sepsis presented fewer DR1s and more DR3s and DR4s. The normal heart rate in healthy infants is always faster than in older children and adults [29]. Bradycardia in infants may be secondary to several causes, e.g. hypoxemia, toxaemia or sepsis [30]. Billois et al. showed for the first time that infants at risk of sepsis express a significant change in the HR microstructure in the form of an increased number of HR decelerations, which appear to group into longer runs at the cost of deceleration singlets [28].

Using the 300-minute ECGs recorded during sleep from seventy-eight patients with suspected obstructive sleep apnoea (OSA), in co-operation with Professor Adrian Baranchuk from Kingston, ON, Canada, we investigated the relationship between the severity of this disease and the asymmetric features of HR microstructure [31]. Patients with severe OSA had fewer DR1s and AR1s than subjects with moderate OSA, and more AR5, AR10s and DR5s, DR8s than patients with no or mild OSA. Additionally, the longest acceleration runs were significantly longer in patients from the severe OSA (on the average of the length 9) than in individuals with no or a mild form of this disease (on average of the length 8). Interestingly, the severity of OSA was not significantly associated with the values of any measures of HRV (including spectral analysis).

The first impression from the OSA study was that it contrasts with the findings from post-infarction patients [26,31]. While high-risk survi-

vors of myocardial infarction had fewer longer runs in the 24-hour ECGs [26], the most severe OSA patients had more such runs in the 300-minute ECG recorded during sleep [31]. Regardless of technical differences, duration of the recording, comparing sleep ECGs vs the whole 24-hour ECGs, one important feature must be underlined, OSA patients, particularly with the more severe form, have many repeated episodes of apnoea and hypopnea during sleep [32,33]. The hypopnea/apnoea episode lasts at least 10 seconds, and patients with severe OSA have at least 30 such incidents during the polysomnographic recording (ECG is one of several vital signals recorded during polysomnography) [33]. In the studied patients, the average number of hypopnea/apnoea episodes was 43 [31]. In OSA patients, after each hypopnea/apnoea, there was increased breathing which, for a short period, was deeper and faster. Typically during hypopnea or apnoea, there is a reduction in HR, bradycardia is common, and even short pauses may appear, when breathing is restored, HR rapidly increases and such erratic breathing strongly influences HR [31,33]. It seems that more episodes of hypopnea or apnoea triggered more HR decelerations, whereas during the consequent increase in breathing more HR accelerations were found. Both HR decelerations and accelerations clustered in longer runs more commonly in patients with more severe OSA [31].

In 2017, Jiang et al. published their study on the HR accelerations and decelerations in a larger group of 231 OSA patients and analysed 6-hour ECGs [34]. They confirmed our observations that individuals with severe OSA had more longer HR acceleration runs (AR2-AR5, and undefined long AR) and deceleration runs (DR5 and undefined long DR). Additionally, they found in a prospective substudy of 39 OSA (8 moderate and 31 severe) patients that a one-night treatment with a continuous positive airway pressure reduced the number of longer ARs and DRs. It was the first-ever observation from a prospective clinical study proving that a planned medical intervention (in this case, the continuous positive airway pressure applied for one night) significantly modified the HR microstructure.

Recently, Kong et al. studied 151 patients with chronic obstructive pulmonary disease (COPD) and 45 patients without COPD [35], reporting that COPD patients had significantly fewer DR2s, DR4s and DR8s than the remaining individuals. Additionally, these authors reported that the numbers of longer DRs were negatively associated with the presence of arrhythmia, i.e. premature atrial beats, supraventricular tachycardias, and premature ventricular beats, that is, reduced numbers of DR2, DR4 and DR8 were associated with a higher risk of cardiac arrhythmias. Unfortunately, there are no data on the deceleration runs of other length or any acceleration runs. However, this study is clinically relevant as it shows the predictive value of the analysis of the HR microstructure and its asymmetry in another group of patients, this time with COPD.

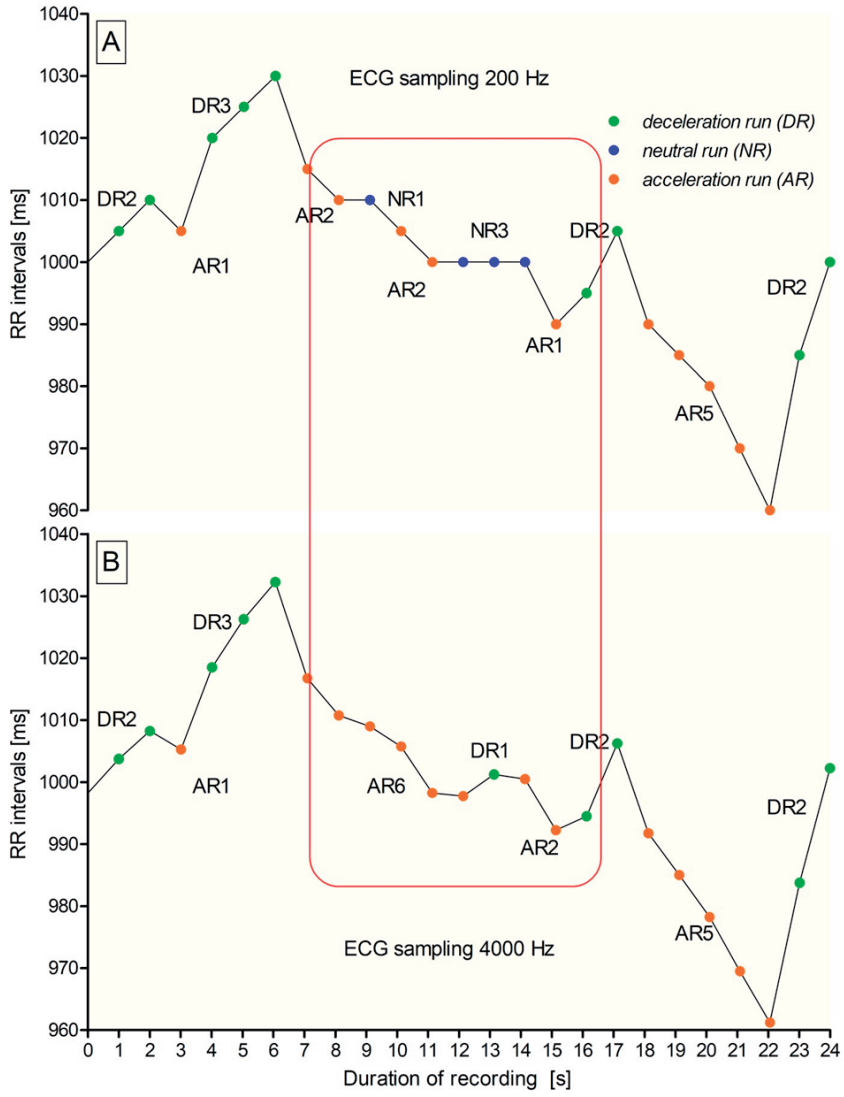
Limitations of the analysis of HR microstructure

The main limitation of counting the number of monotonic runs is the length of the recording, as the probability of occurrence of longer runs is low in short recordings lasting only a couple of minutes. For this reason, longer runs, particularly very long runs of the length of over 12 and more consecutive RR intervals changing in the same direction are easier to spot in ECG recordings of several hours duration [20,25]. Thus, for ECG recordings lasting up to 5 or 10 minutes, it is better to limit the analysis of HR microstructure to shorter runs up to 4 beats. For ECGs of moderate length such as up to 30–60-minute recordings, the analysis of the monotonic runs should be limited to 8 beats. For the 24-hour ECGs, it is common to find longer monotonic runs between 12 and 16 beats, even of the length of 24 beats [20,25]. As far as we know, no data exist with the HR microstructure analysis in ECG recordings extending for more than 24 hours.

Another limitation is the sampling frequency of the recorded ECG. Previous studies on the microstructure of HRA used a lower sampling frequency of 200 Hz for the 24-hour Holter ECGs, which translates into the precision of RR intervals of 5 ms [20,25]. Consequently, there was a substantial number of so-called neutral runs (up to 6–7% of all beats), i.e., such consecutive RR intervals which have identical duration. For instance, the neutral run of three (NR3) is composed of four successive and equal RR intervals with the first as

the reference for the 2nd, the 2nd for the 3rd, and the 3rd for the 4th RR interval. RR intervals before the first and the fourth RR intervals must be different. In total, the number of comparisons showing no change for this particular neutral run equals three. The following time series of RR intervals is more explanatory: 1000 ms (1st RR interval), 1000 ms (2nd RR interval), 1000 ms (3rd RR interval), and 1000 ms (4th RR interval) (**Figure 4**).

In the study of post-infarction patients, the sampling frequency of the 24-hour ECGs was even lower, 128 Hz [26]. The same sampling frequency was applied ECGs from OSA patients [31]. However, none of these studies revealed the number of neutral runs. The information on the sampling frequency and the number of neutral runs should be reported in all studies on the HR microstructure.



200 Hz ECG sampling	1015	1010	1010	1005	1000	1000	1000	1000	990	995
		A	N	A	A	N	N	N	A	D
4000 Hz ECG sampling	1016,75	1010,75	1009,00	1005,75	998,25	997,75	1001,25	1000,50	992,25	994,50
		A	A	A	A	A	D	A	A	D

Figure 4. Effect of sampling frequency on the measurement of RR intervals and their labelling. The upper panel shows a local tachogram for the ECG recorded at the sampling frequency of 200 Hz whereas the lower panel presents the same ECG recorded at the sampling frequency of 4000 Hz. In the upper panel, there are neighbouring RR intervals of identical duration (marked with blue dots), so-called neutral runs (NR), and two examples of such runs are presented as NR1 and NR3. The lower panel, however, shows that these RR intervals did not have the same duration, and instead of NRs, there were some HR accelerations (ARs) and decelerations (DRs). A higher sampling frequency of the recorded ECG limits the number of falsely identified NR, providing a proper number of acceleration and deceleration runs. The bottom panel shows the values of RR intervals with the corresponding labelling for both types of ECG sampling

Newer long-term recorders have a much higher sampling frequency of ECG between 1000 Hz to 4000 Hz, which may translate to the identification of RR intervals with the precision between 1 to 0.25 ms. However, due to the intrinsic properties of the ECG signal, such high frequency might increase the signal-to-noise ratio. Additionally, at higher sampling frequencies, the peak of R wave flattens and no longer resembles a point. Taken together, an increase in the noise and flattening of the peaks of R waves might interfere with the proper measurement of the distances of RR intervals. Although it is tempting to believe that higher sampling frequencies might improve the precision of RR intervals measurement and limit or even zero the number of neutral runs, this is uncertain and requires separate studies. However, the currently applied devices record ECG at a much lower sampling frequency between 125 and 250 Hz, so there is room for the improvement of RR intervals measurement. In effect, the analysis of the asymmetric properties of HR microstructure should be more accurate and increase the number of adequately detected longer DRs and ARs. As discussed in a previous study [20], the neutral runs seem to be an artificial effect of low sampling frequency than a genuine physiological phenomenon. Studying the distribution of acceleration and deceleration runs in Holter ECGs recorded at a much higher frequency than 125–250 Hz [20] might result in a lower number of or even no neutral runs, and more precise description of the asymmetric features of HR microstructure.

In summary, the analysis of the HR microstructure and its asymmetric properties seems to be attractive from both a physiological and clinical point of view. It helps to visualise how the RR time series are distributed and to understand how HRV is created. Studying the HR microstructure in clinical scenarios has been demonstrated not only to have predictive value in certain groups of patients but also to monitor the effects of some interventions (e.g., the application of continuous positive airway pressure in OSA patients). In contrast to many other methods transferred to HRV from different scientific areas like astronomy, economy or physics, the monotonic runs methods were deliberately developed to study specific features of normal heart rate and further explore HRA [12,13,15]. We believe that this method deserves more attention and requires further investigation.

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

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References

1. Tomaselli GF, Rubart M, Zipes DP. Mechanisms of Cardiac Arrhythmias. In: Braunwald's Heart Disease: A Textbook of Cardiovascular Medicine. 10th Edition. Saunders; 2014:619-49.
2. Fedorov VV, Glukhov AV, Chang R, Kostecki G, Aferol H, Hucker WJ, Wuskell JP, Loew LM, Schuessler RB, Moazami N, Efimov IR. Optical Mapping of the Isolated Coronary-Perfused Human Sinus Node. Journal of the American College of Cardiology. 2010 Oct;56(17):1386-1394. https://doi.org/10.1016/j.jacc.2010.03.098
3. Shaffer F, McCraty R, Zerr CL. A healthy heart is not a metronome: an integrative review of the heart's anatomy and heart rate variability. Frontiers in Psychology. 2014 Sep 30;5. https://doi.org/10.3389/fpsyg.2014.01040 PMID 25324790
4. Opthof T. The normal range and determinants of the intrinsic heart rate in man. Cardiovascular Research. 2000 Jan 1;45(1):177-184. https://doi.org/10.1016/s0008-6363(99)00322-3
5. Sanchez-Quintana D. Sinus node revisited in the era of electroanatomical mapping and catheter ablation. Heart. 2005 Feb 1;91(2):189-194. https://doi.org/10.1136/hrt.2003.031542
6. Moïse NS, Flanders WH, Pariaut R. Beat-to-Beat Patterning of Sinus Rhythm Reveals Non-linear Rhythm in the Dog Compared to the Human. Frontiers in Physiology. 2020 Jan 22;10. https://doi.org/10.3389/fphys.2019.01548 PMID 32038271
7. Boineau JP, Canavan TE, Schuessler RB, Cain ME, Corr PB, Cox JL. Demonstration of a widely distributed atrial pacemaker complex in the human heart.. Circulation. 1988 Jun;77(6):1221-1237. https://doi.org/10.1161/01.cir.77.6.1221
8. Ho SY, Sánchez-Quintana D. Anatomy and pathology of the sinus node. Journal of Interventional Cardiac Electrophysiology. 2015 Aug 30;46(1):3-8. https://doi.org/10.1007/s10840-015-0049-6
9. Peters CH, Sharpe EJ, Proenza C. Cardiac Pacemaker Activity and Aging. Annual Review of Physiology. 2020 Feb 10;82(1):21-43. https://doi.org/10.1146/annurev-physiol-021119-034453
10. Boyett M. The sinoatrial node, a heterogeneous pacemaker structure. Cardiovascular Research. 2000 Sep;47(4):658-687. https://doi.org/10.1016/s0008-6363(00)00135-8
11. John RM, Kumar S. Sinus Node and Atrial Arrhythmias. Circulation. 2016 May 10;133(19):1892-1900. https://doi.org/10.1161/circulationaha.116.018011
12. Electrophysiology TFOTES. Heart Rate Variability. Circulation. 1996 Mar;93(5):1043-1065. https://doi.org/10.1161/01.cir.93.5.1043

13. Sassi R, Cerutti S, Lombardi F, Malik M, Huikuri HV, Peng C, Schmidt G, Yamamoto Y, Gorenek B, Lip GY, Grassi G, Kudaiberdieva G, Fisher JP, Zabel M, Macfadyen R. Advances in heart rate variability signal analysis: joint position statement by the e-Cardiology ESC Working Group and the European Heart Rhythm Association co-endorsed by the Asia Pacific Heart Rhythm Society. Europace. 2015 Jul 14;17(9):1341-1353. https://doi.org/10.1093/europace/euv015
14. Berntson GG, Cacioppo JT, Quigley KS. Respiratory sinus arrhythmia: Autonomic origins, physiological mechanisms, and psychophysiological implications. Psychophysiology. 1993 Mar;30(2):183-196. https://doi.org/10.1111/j.1469-8986.1993.tb01731.x
15. Piskorski J, Guzik P, Krauze T, Żurek S. Cardiopulmonary resonance at 0.1 Hz demonstrated by averaged Lomb-Scargle periodogram. Open Physics. 2010 Jan 1;8(3). https://doi.org/10.2478/s11534-009-0101-1
16. Guzik P, Piskorski J, Krauze T, Wykretowicz A, Wysocki H. Heart rate asymmetry by Poincaré plots of RR intervals. Biomedizinische Technik/Biomedical Engineering. 2006 Oct;51(4):272-275. https://doi.org/10.1515/bmt.2006.054
17. Piskorski J, Guzik P. Geometry of the Poincaré plot ofRRintervals and its asymmetry in healthy adults. Physiological Measurement. 2007 Feb 19;28(3):287-300. https://doi.org/10.1088/0967-3334/28/3/005
18. Guzik P, Piskorski J, Krauze T, Wykretowicz A, Wysocki H. Partitioning total heart rate variability. International Journal of Cardiology. 2010 Sep;144(1):138-139. https://doi.org/10.1016/j.ijcard.2008.12.151
19. Piskorski J, Guzik P. Asymmetric properties of long-term and total heart rate variability. Medical & Biological Engineering & Computing. 2011 Sep 28;49(11):1289-1297. https://doi.org/10.1007/s11517-011-0834-z
20. Piskorski J, Guzik P. The structure of heart rate asymmetry: deceleration and acceleration runs. Physiological Measurement. 2011 Jun 7;32(8):1011-1023. https://doi.org/10.1088/0967-3334/32/8/002
21. Piskorski J, Guzik P. Compensatory properties of heart rate asymmetry. Journal of Electrocardiology. 2012 May;45(3):220-224. https://doi.org/10.1016/j.jelectrocard.2012.02.001
22. Mieszkowski D, Kośmider M, Krauze T, Guzik P, Piskorski J. Asymmetric detrended fluctuation analysis reveals asymmetry in the RR intervals time series. Journal of Applied Mathematics and Computational Mechanics. 2016 Mar;15(1):99-106. https://doi.org/10.17512/jamcm.2016.1.10
23. Piskorski J, Kosmider M, Mieszkowski D, Krauze T, Wykretowicz A, Guzik P. Properties of Asymmetric Detrended Fluctuation Analysis in the time series of RR intervals. Physica A: Statistical Mechanics and its Applications. 2018 Feb;491:347-360. https://doi.org/10.1016/j.physa.2017.09.057
24. Piskorski J, Ellert J, Krauze T, Grabowski W, Wykretowicz A, Guzik P. Testing heart rate asymmetry in long, nonstationary 24 hour RR-interval time series. Physiological Measurement. 2019 Oct 30;40(10):105001. https://doi.org/10.1088/1361-6579/ab42d5
25. Piskorski J. Struktura asymetrii rytmu serca (Structure of heart rate asymmetry). Poznań: Wydawnictwo Naukowe; 2011.
26. Guzik P, Piskorski J, Barthel P, Bauer A, Müller A, Junk N, Ulm K, Malik M, Schmidt G. Heart rate deceleration runs for postinfarction risk prediction. Journal of Electrocardiology. 2012 Jan;45(1):70-76. https://doi.org/10.1016/j.jelectrocard.2011.08.006
27. Guzik P, Nieminen T, Piskorski J, Kaiser W, Viik J, Nikus K, Lehtinen R, Lehtimäki T, Kähönen M. Increased rate of fast-changing microstructure of heart rate asymmetry predicts mortality in patients undergoing a clinically indicated exercise test. Cardiol. J. 2011;18:593.
28. Billois R, Porée F, Beuchée A, Carrault G. Interest of RR deceleration for diagnosis of late onset sepsis. In: IEEE Computing in Cardiology. Kraków; 2012:633-6.
29. Fleming S, Thompson M, Stevens R, Heneghan C, Plüddemann A, Maconochie I, Tarassenko L, Mant D. Normal ranges of heart rate and respiratory rate in children from birth to 18 years of age: a systematic review of observational studies. The Lancet. 2011 Mar;377(9770):1011-1018. https://doi.org/10.1016/s0140-6736(10)62226-x PMID 21411136
30. Baruteau A, Perry JC, Sanatani S, Horie M, Dubin AM. Evaluation and management of bradycardia in neonates and children. European Journal of Pediatrics. 2016 Jan 16;175(2):151-161. https://doi.org/10.1007/s00431-015-2689-z
31. Guzik P, Piskorski J, Awan K, Krauze T, Fitzpatrick M, Baranchuk A. Obstructive sleep apnea and heart rate asymmetry microstructure during sleep. Clinical Autonomic Research. 2013 Jan 24;23(2):91-100. https://doi.org/10.1007/s10286-013-0188-8
32. Bibbins-Domingo K, Grossman DC, Curry SJ, Davidson KW, Epling JW, García FAR, Herzstein J, Kemper AR, Krist AH, Kurth AE, Landefeld CS, Mangione CM, Phillips WR, Phipps MG, Pignone MP, Silverstein M, Tseng C. Screening for Obstructive Sleep Apnea in Adults. JAMA. 2017 Jan 24;317(4):407. https://doi.org/10.1001/jama.2016.20325
33. Parati G, Lombardi C, Hedner J, Bonsignore MR, Grote L, Tkacova R, Levy P, Riha R, Bassetti C, Narkiewicz K, Mancia G, McNicholas WT. Position paper on the management of patients with obstructive sleep apnea and hypertension. Journal of Hypertension. 2012 Apr;30(4):633-646. https://doi.org/10.1097/hjh.0b013e328350e53b
34. Jiang J, Chen X, Zhang C, Wang G, Fang J, Ma J, Zhang J. Heart rate acceleration runs and deceleration runs in patients with obstructive sleep apnea syndrome. Sleep and Breathing. 2016 Nov 23;21(2):443-451. https://doi.org/10.1007/s11325-016-1437-6
35. Kong Z, Wang X, Shen S, Liu H, Zhou L, Chen B, Chen Z, Yin S. Risk Prediction for Arrhythmias by Heart Rate Deceleration Runs in Patients with Chronic Obstructive Pulmonary Disease. International Journal of Chronic Obstructive Pulmonary Disease. 2020 Mar;Volume 15:585-593. https://doi.org/10.2147/copd.s234470

REVIEW PAPER

JMS Journal of Medical Science

Drug design: 4-thiazolidinones applications. Part 2. Pharmacological profiles

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Keywords: Structure-based drug design, 5-Ene-4--thiazolidinones, Thiopyrano[2,3-d]thiazoles, biological activity, SAR analysis, Michael acceptors

ABSTRACT

Following the chemical diversity of 4-thiazolidinones, an in-house library of new heterocycles was designed and synthesised (more 7000 compounds). Anticancer, antitrypanosomal, antituberculosis and antiviral activity screening led to the SAR database formation, lead-compound identification, design of focussed sub-libraries, as well as the formation and validation of hypotheses for structure optimisation: i) complications of C5 fragment and/or functionalisation of N3 position; ii) creation of the hybrid molecules; iii) fixation of 5-ene-4-thiazolidinones in fused heterocycles via annulation (thiopyrano[2,3-d]thiazoles were found as cyclic isosteric mimetics of 5-ene-4-thiazolidinones); iv) the leukaemia panel was detected to be the most sensitive among all cancer cell lines. The subsequent in silico and pharmacological data obtained in the investigation of the molecular mechanism of the anticancer effect revealed the apoptotic-related and mild prooxidant actions of the active compounds.

The main milestones of the project involved synthetic investigations, biological activity studies, rational approaches (QSAR-analysis, docking, molecular modelling etc.) to "drug-like" molecule design [1–3]. Initially, various activity types were studied (anti-inflammatory [4, 5], antimicrobial [6], anticonvulsant [7], choleretic [8], etc.), then investigations focussed on the study of anticancer, antimycobacterial, antiviral and antitrypanosomal activities. More than 2000 biological assays were conducted, allowing the identification of at least 200 hit compounds with anticancer action, 40 hits with antimycobacterial activity and 30 hits with the inhibitory activity against different virus strains. The early stage results demonstrated that the compounds possessed anti-inflammatory activity, antimicrobial action, antioxidant, choleretic and anticonvulsant activities.

Anticancer activity

The in-house library of heterocycles has been an object for the study of anticancer activity within the NCI, NIH protocol [9–11]. Among the 1,750 tested compounds, 525 (30.0%) have successfully passed the pre-screening phase (**Figure 2**). After passing the second testing phase, 14 compounds were submitted for consideration by the NCI Biological Committee, among them, 8 compounds were affirmed for in-depth *in vivo* preclinical trials as potential anticancer agents.

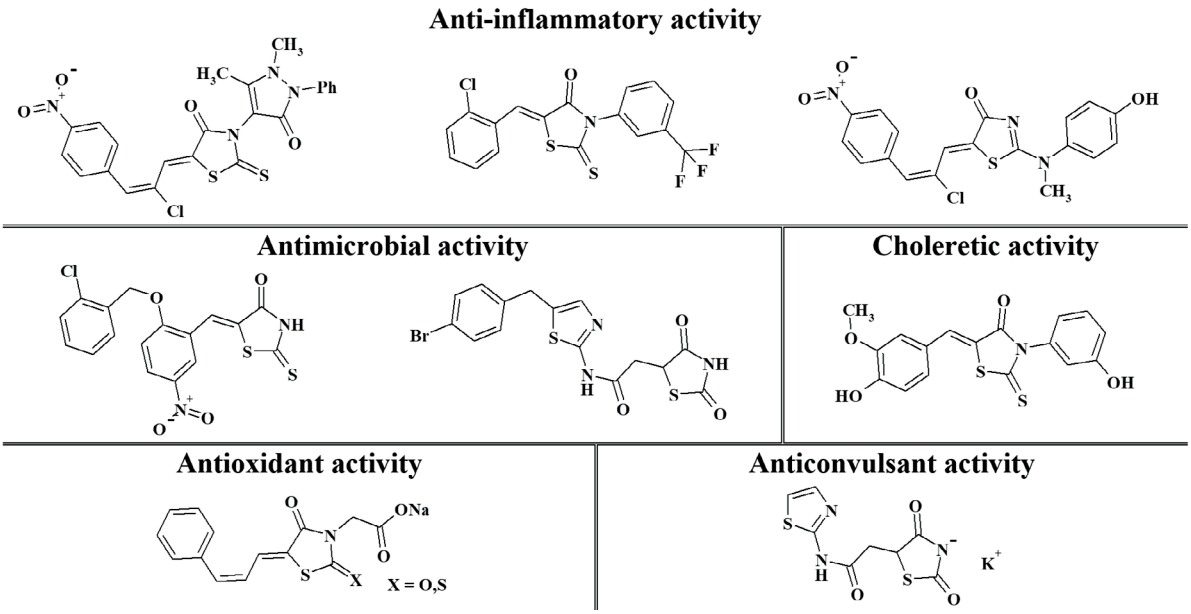


Figure 1. Hit compounds identified in the early stage of the project

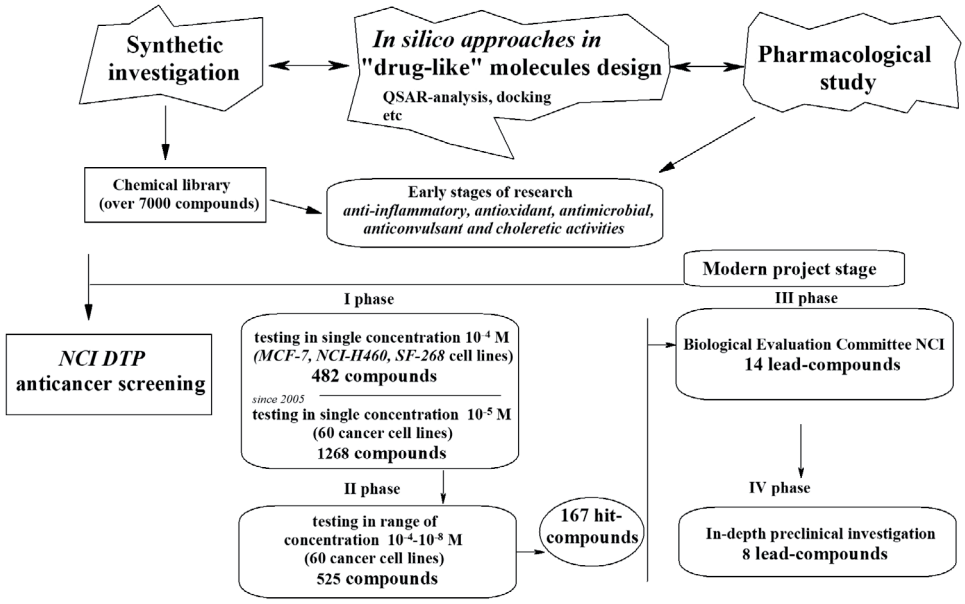


Figure 2. Scheme of the Department of Pharmaceutical, Organic and Bioorganic Chemistry project design

Figure 3 presents selected hit compounds from different groups that possess high antimitotic effect *in vitro* at submicromolar concentrations (10^{-5}–10^{-8} M) and are characterised by low *in vivo* toxicity. It is important to note that these compounds are representative of 5-ene-4-thiazolidinones ("Biological way") [12–25] and thiopyrano[2,3-*d*]thiazoles ("Chemical way") [36–30].

Interestingly, in the anticancer selectivity rating, the leukaemia cell lines were the most sensitive to 4-thiazolidinones and related heterocyclic systems following the analysis of the in-depth *in vitro* research results. A series of cell lines, such as leukaemia lines (*CCRF-CEM, HL-60(TB), RPMI-8226, SR, K-562, MOLT-4*), CNS cancer line (*U251*), non-small cell lung cancer line (*HOP-92*), renal cancer cell lines (*UO-31*, *786-O*), colon cancer line (*HCT-116*) as well as breast cancer line (*MDA-MB 231*) were found to be the most sensitive to the test compounds. Thus, based on the obtained results, it was hypothesised that the heterocycles containing a "thiazolidinone matrix" have specific anti-leukaemia activity.

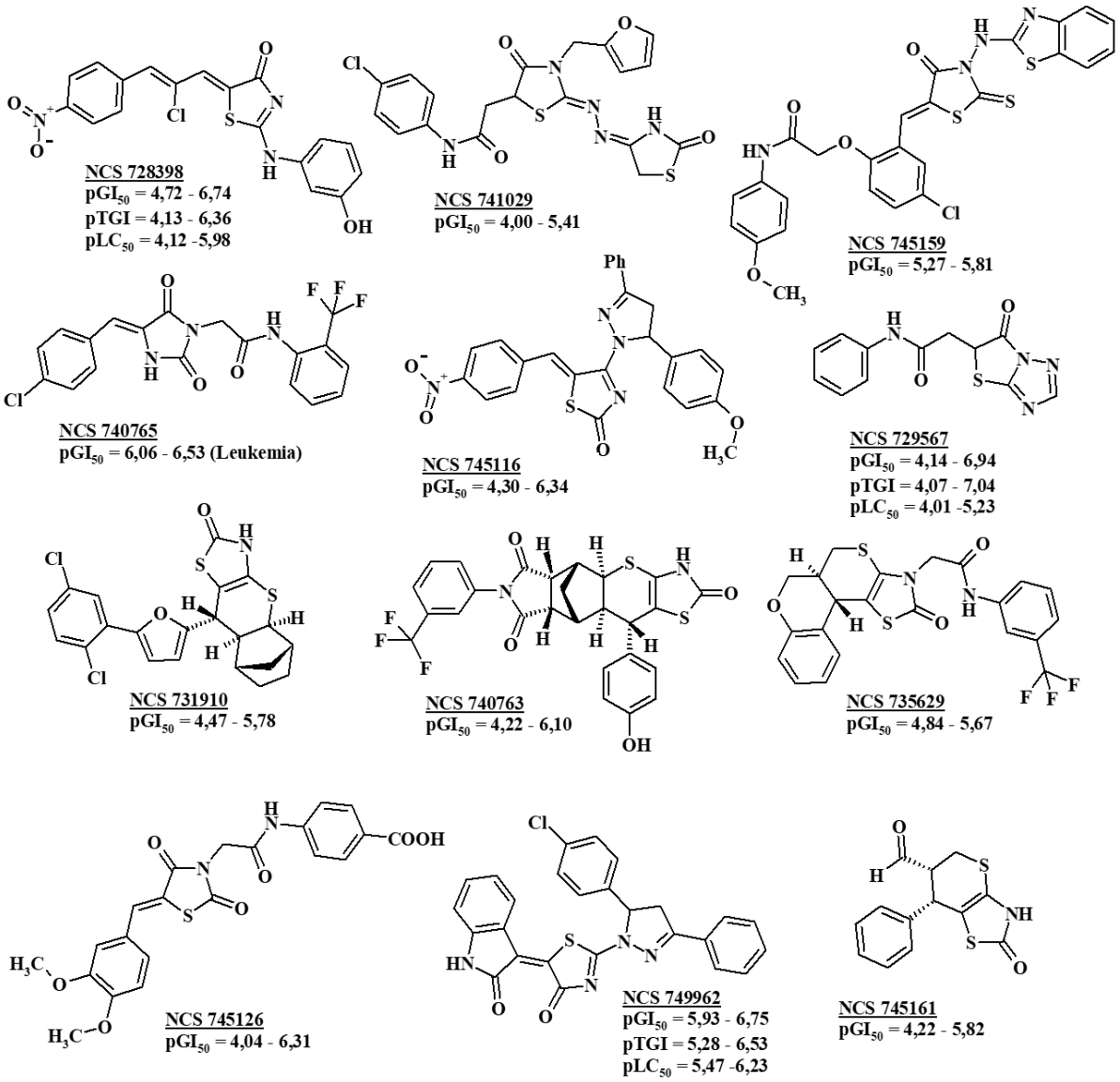


Figure 3. Hit compounds from different groups of 4-thiazolidinone derivatives with a high antimitotic effect *in vitro*

In silico approaches for anticancer activity data analysis. The COMPARE analysis [10,11] was performed for the active compounds to investigate the similarity of their cytotoxicity pattern (mean graph fingerprints) with those of known anticancer standard agents, NCI active synthetic compounds and natural products. For some synthesised heterocyclic substances, there was established correlation with the inhibitors of tubulin polymerisation, RNA polymerase, p-glycoprotein or topoisomerase II, inductors of apoptosis and activators of caspases. It is of note that the significant values of the correlation coefficients of thiazolidinone derivatives from different sub-libraries to the S-trityl-L-cysteine, aminoacyl-tRNA synthetases inhibitor with antiproliferative effect against leukaemia.

Following the analysis of anticancer activity profiles of different thiazolidinones using modern computational methods, like principal components analysis, neutral networks and cluster analysis, it was found that two different mechanisms and a "mixed" mechanism were responsible for the anticancer activity [3, 31–33].

In cooperation with the Institute of Cell Biology, NAS of Ukraine (Prof Rostyslav Stoika), the hypothesis regarding the apoptosis-dependent mechanism of antitumor activity was confirmed. Moreover, the proapoptotic activity of the tested compounds was observed. Studies have been

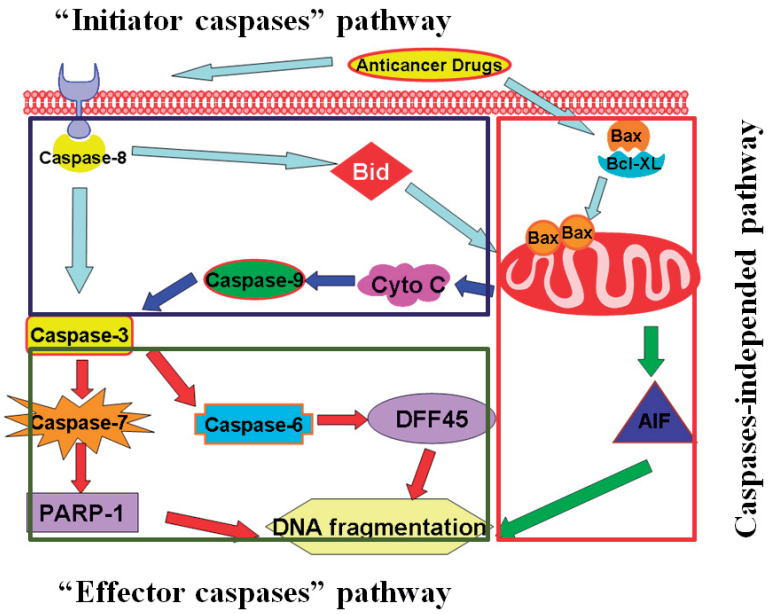


Figure 4. Plausible mechanisms of the anticancer effect of 5-ene-4-thiazolidinones

conducted regarding the effects of compounds on some cytokines, in particular, initiatory effector groups of caspases and cytokines involved in the development of caspase-independent apoptosis. Different apoptotic-related pathways were detected. Also, it should be noted that there were various implementations of such an effect, involving the "classic" apoptotic pathway (mediated by Bax & Caspase-7), caspase-independent apoptosis (mediated by AIF) and "mixed-type" apoptosis (mediated by AIF, Bax & Caspase-9) (**Figure 4**) [34–37]. This data correlated well with the results obtained in in silico studies [3, 31–33].

Based on the analysis of the biological and *in silico* data, we proposed a pharmacophore model for the design of potential anticancer agents [38, 39]. The pharmacophore (**Figure 5**) consists of two aromatic or π-ring system centres, a hydrophobic group and the two projections of the hydrogen bond donors (electron pair acceptors) (error rate 0.8%, accuracy 87.5% and precision = 99.5%).

A new sub-library was created (690 structures) by varying the substituents in the 2,4 and 5 positions of 4(2)-thiazolidinone. Pharmacophore and Random Forest models predicted 101 and 32 hit compounds from the virtual sub-li-

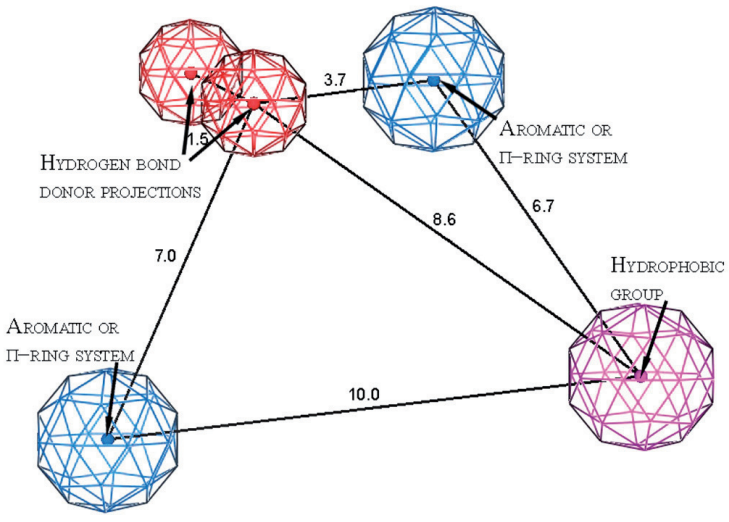


Figure 5. Probable pharmacophore model of 4-thiazolidinones anticancer activity

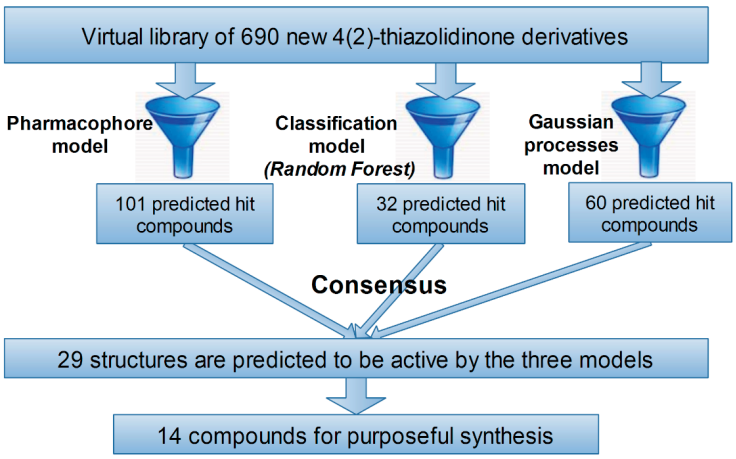
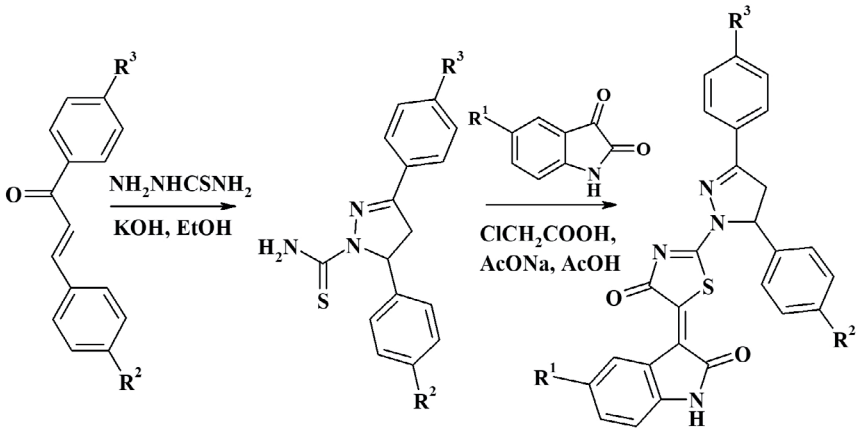


Figure 6. Virtual screening scheme

Scheme 1



92. R^1=H, R^2=R^3=Cl,
93. R^1=R^2=R^3=Cl,
94. R^1=Br, R^2=R^3=Cl,
95. R^1=H, R^2=Cl, R^3=Br,
96. R^1=R^2=Cl, R^3=Br,
97. R^1=R^3=Br, R^2=Cl,
98. R^1=H, R^2=Cl, R^3=F,
99. R^1=R^2=Cl, R^3=F,
100. R^1=Br, R^2=Cl, R^3=F,
101. R^1=H, R^2=NMe_2, R^3=Cl,
102. R^1=R^3=Cl, R_2=NMe_2,
103. R^1=Br, R^2=NMe_2, R^3=Cl,
104. R^1=H, R^2=NMe_2, R^3=Br,
105. R^1=Cl, R^2=NMe_2, R^3=Br

brary, respectively. Analysis of the distances to the model showed that all predicted compounds were active, falling into the applicability domain, while 47 out-of-domain compounds were inactive. The Gaussian processes model predicted the cytotoxic effect on tumour cells for 60 structures. The choice of virtual screening hits was based on the consensus between all predictions, thereby predicting 29 hit compounds using these three screening models, of which, 14 structures were selected for synthesis (**Figure 6**).

Using the above-mentioned approach, 14 novel derivatives **92**-**105** were selected and synthesised (**Scheme 1**).

The success of the purposeful strategy was confirmed by biological assays, according to which, the synthesised compounds inhibited cancer cell growth, even at micromolar concentrations. The four most potent synthesised compounds **94**, **96**, **97** and **103** showed IC_{50} values between 0.16–10 μM in MTT assays of rat glioma cells C6, Mino cells, Jurkat and L1210 cells.

Antituberculosis activity

The study of antimicrobial activity was conducted on the Mycobacterium tuberculosis H37Rv (ATCC 27294) within the Tuberculosis Antimicrobial Acquisition & Coordination Facility (TAACF) Programme at the National Institute of Allergic and Infectious Diseases (NIAID, USA). Among the tested compounds, 40 active substances with an IC_{90} ≤10 μg/mL were identified, for which the cytotoxicity of mammalian cells (CC_{50}) was determined on VERO cells and a selectivity index (SI = CC_{50}/IC_{90}) was calculated. For the in-depth studies, 7 derivatives (**Figure 7**) from different sub-libraries (5-ene-4-thiazolidinones and thiopyrano[2,3-*d*] thiazoles) were selected.

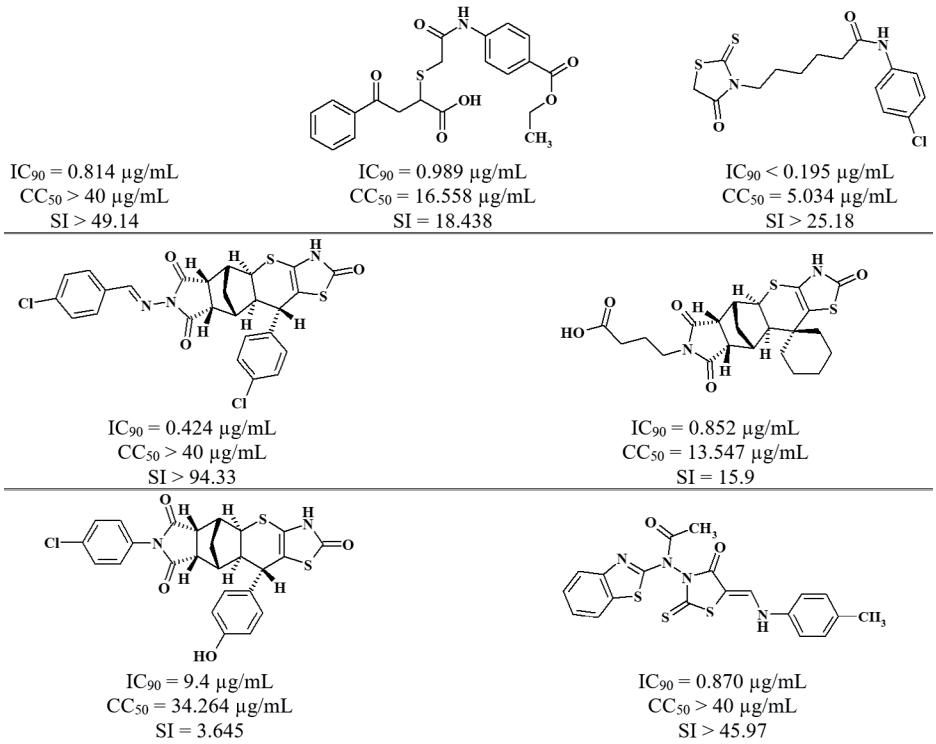


Figure 7. Hit compounds with antituberculosis activity

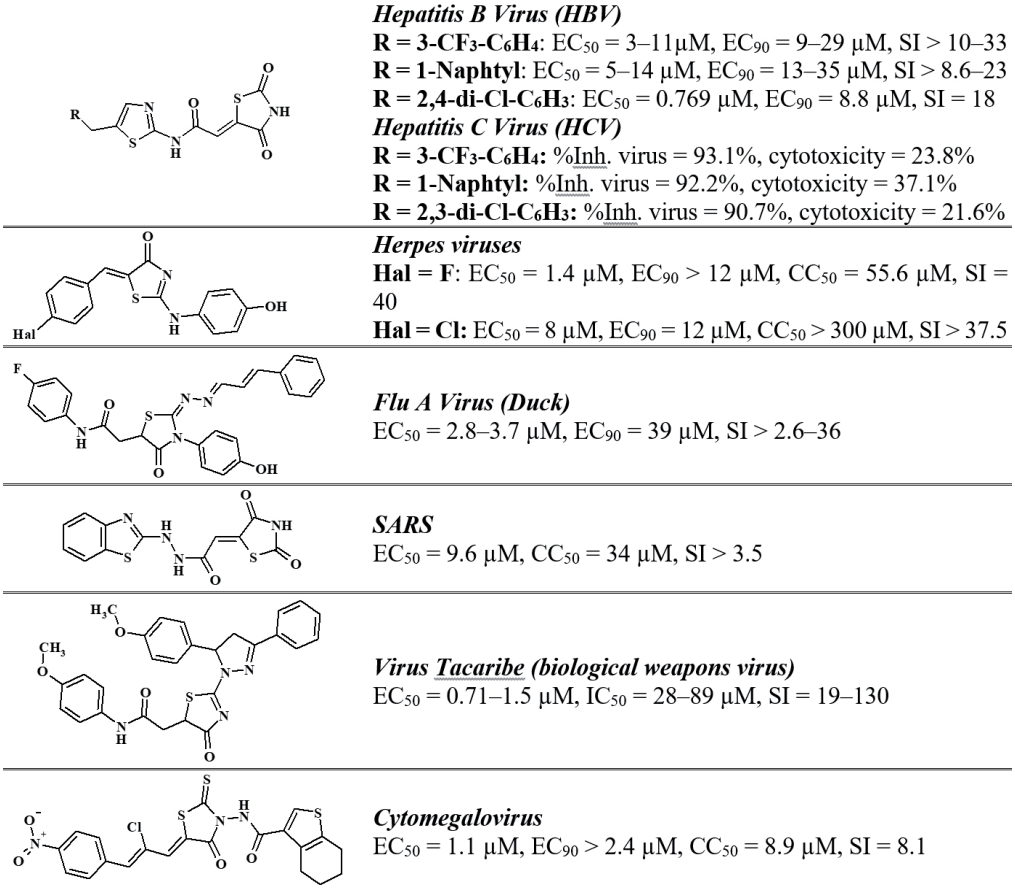


Figure 8. Hit compounds with antiviral activity

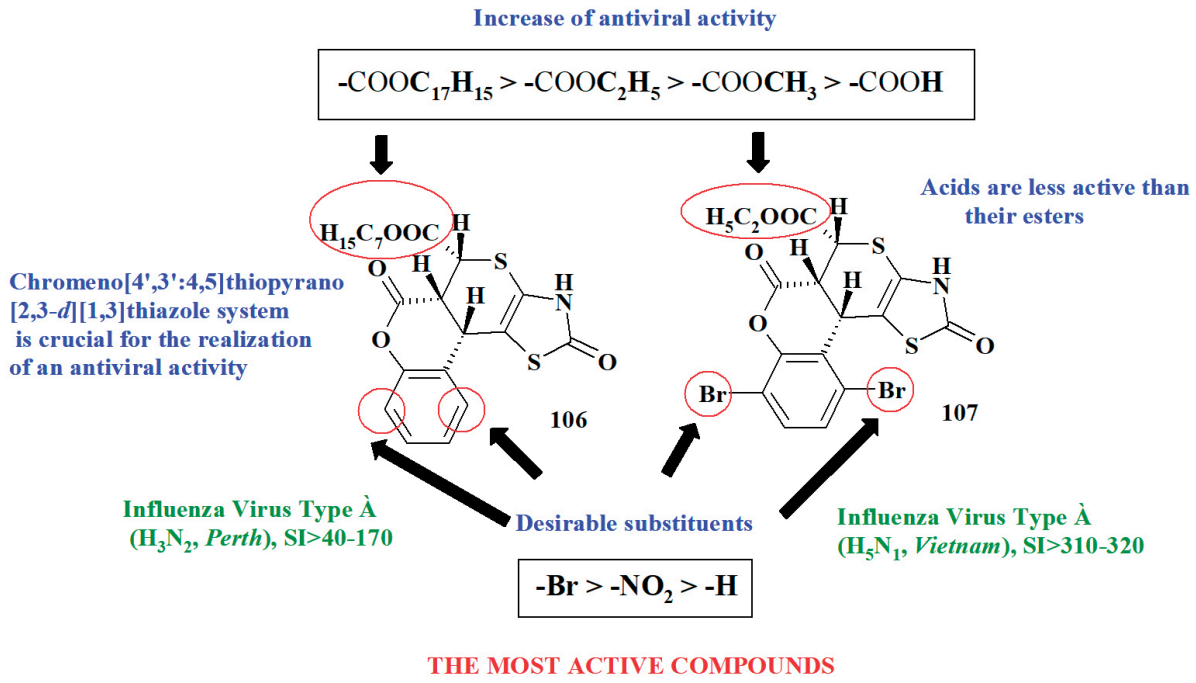
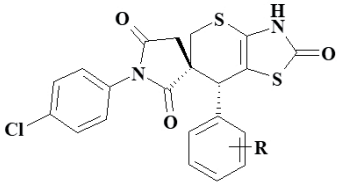


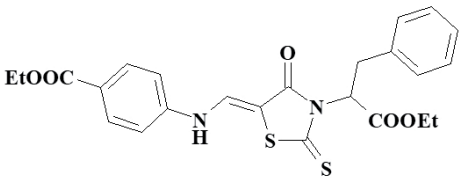
Figure 9. SAR study of thiopyrano[2,3-*d*][1,3]thiazoles

Thiopyrano[2,3-d]thiazoles



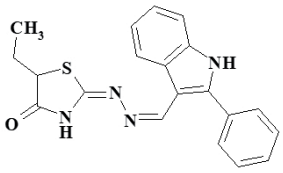
R = 3,4-(OMe)2
IC_{50} = 0.26 μM (*TBB*), 0.42 μM (*TBG*)
R = 4-NMe2
IC_{50} = 1.26 μM (*TBB*), 1.46 μM (*TBG*)
R = 4-NEt2
IC_{50} = 2.39 μM (*TBB*), 0.93 μM (*TBG*)

5-Enamine-4-thiazolidinones



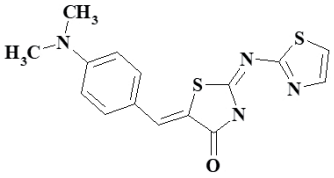
IC_{50} = 0.091 μM (*TBB*), 0.027 μM (*TBG*)

4-Thiazolidinone-hydrazones



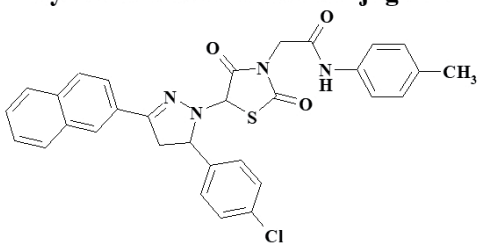
IC_{50} = 0.03 μM (*TBB*), 0.11 μM (*TBG*)

2-Hetarylimino-4-thiazolidinones



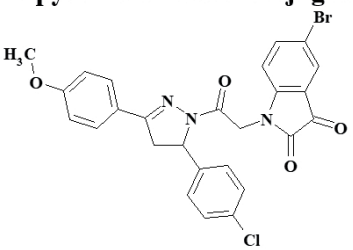
IC_{50} = 1.09 μM (*TBB*), 1.09 μM (*TBG*)

Pyrazoline-thiazolidine conjugates



IC_{50} = 2.53 μM (*TBB*), 5.43 μM (*TBG*)

The pyrazoline-isatin conjugates



IC_{50} = 1.49 μM (*TBB*), 0.76 μM (*TBG*)

Figure 10. Hit compounds with pronounced antitrypanosomal activity

Antiviral activity

Screening of the antiviral activity of the synthesised compounds was also conducted within the Antimicrobial Acquisition & Coordination Facility (AACF, NIAID, USA) Programme. As a result, 30 compounds were identified with significant antiviral activity and sufficient selectivity indices [26, 40–43]. In addition, a group of 4-thiazolidinone-related compounds (**Figure 8**) with a strong effect on hepatitis B (HBV) and C (NSV) and Flu A & B viruses were identified, as well as one high-level SARS and Tacaribe strain (biological weapons virus).

Derivatives of *rel*-(5*R*,5a*R*,11b*S*)-2,6-dioxo-3,5a,6,11b-tetrahydro-2*H*,5*H*-chromeno[4',3':4,5] thiopyrano[2,3-*d*][1,3]thiazole-5-carboxylic acids belong to the most promising group of compounds (**Figure 9**). The substituents in the positions 8 and 10 and the ester group in position 5 are desirable for antiviral activity. The increase of the alkyl moiety length leads to an increased effect, thus, compound **106** showed a higher activity against Influenza Virus Type A (H_3N_2, Perth strain) with an EC_{50} = 0.6 ÷ 2.5 μg/ml and SI = 40 ÷> 170 than derivative **107** with an EC_{50} = 0.31 ÷ 0.32 μg/ml and SI => 310 ÷> 320 [42].

The study of antitrypanosomal activity was conducted at the National Museum of Natural History (Prof Philippe Grellier, France) on *Trypanosoma brucei brucei (TBB)* and *Trypanosoma brucei gambiense (TBG)* strains. Among more than 200 tested compounds, a series of hits with pronounced antiparasitic activity in cellulo with IC_{50} values of 0.03–15.64 μg/ml were identified (**Figure 10**) [27, 43–48].

Conclusions and further perspectives

- 4-Thiazolidinones possess a variety of biological activities, both in screening campaigns and directed experiments, hence are considered as a tool for the synthesis of related heterocycles, simplified analogues and diversity complex heterocycles within various approaches.
- Among the variety of thiazolidinone subtypes, 5-ene-thiazolidinones are of special interest as hit- and lead-compounds possessing anticancer, antimicrobial, antiviral, and antitrypanosomal activities.
- Assigning 5-ene-thiazolidinones as pan assay interference compounds (PAINS) due to their possible Michael acceptor functionality must be analysed in-depth: experimental confirmation is essential to claim target compounds as PAINS; the positive aspect of such covalent modifiers should not be discarded.
- Annealing of a thiazolidine core into thiopyranothiazole analogues is one of prominent optimisation directions which will allow conservation of the activity pattern of synthetic precursors (5-ene-4-thiazolidinones), decrease the toxicity and avoid the Michael acceptor properties.
- The main directions for 4-thiazolidinones optimisation are: *i*) complication of the fragment in the C5 position; *ii*) introduction of the substituents in the N3 position (especially fragments with carboxylic group or its derivatives); *iii*) annealing in complex heterocyclic systems; *iv*) combination with other pharmacologically attractive fragments within a hybrid pharmacophore approach.
- 4-Thiazolidinones are useful tools for medicinal chemistry and should not be regarded as useless *per se*.

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

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References

1. Lesyk RB, Zimenkovsky BS, Kaminskyy DV, Kryshchyshyn AP, Havryluk DY, Atamanyuk DV, Subtel'na IY, Khyluk DV. Thiazolidinone motif in anticancer drug discovery. Experience of DH LNMU medicinal chemistry scientific group. Biopolymers and Cell. 2011 Mar 20;27(2):107-117. https://doi.org/10.7124/bc.000089
2. Devinyak O, Zimenkovsky B, Lesyk R. Biologically Active 4-Thiazolidinones: A Review of QSAR Studies and QSAR Modeling of Antitumor Activity. Current Topics in Medicinal Chemistry. 2013 Mar 1;12(24):2763-2784. https://doi.org/10.2174/1568026611212240006
3. Devinyak O, Havrylyuk D, Zimenkovsky B, Lesyk R. Computational Search for Possible Mechanisms of 4-Thiazolidinones Anticancer Activity: The Power of Visualization. Molecular Informatics. 2014 Mar;33(3):216-229. https://doi.org/10.1002/minf.201300086

4. Lesyk R, Vladzimirska O, Zimenkovsky B, Golota S, Nektgayev I, Cherpak O, Leb'yak M, Kozak O. Synthesis and antiinflammatory activity of novel 3-(2,3-dimethyl-1-phenyl-4-pyrazolon-5-yl)-4-thiazolidones. Boll Chim Farm. 2002 May-Jun;141(3):197-201. PMID 12197418
5. Lesyk R, Vladzimirska O, Zimenkovsky B, Horishny V, Nektegayev I, Solyanyk V, Vovk O. New thiazolidones-4 with pyrazolone-5 substituent as the potential NSAIDs. Boll Chim Farm. 1998 Jun;137(6):210-7. PMID 9713155
6. Zimenkovskii BS, Kutsyk RV, Lesyk RB, Matyichuk VS, Obushak ND, Klyufinska TI. Synthesis and antimicrobial activity of 2,4-dioxothiazolidine-5-acetic acid amides. Pharmaceutical Chemistry Journal. 2006 Jun;40(6):303-306. https://doi.org/10.1007/s11094-006-0115-6
7. Lesyk R, Pinyazhko O, Myronenko S, Nektegayev I. 5-(3-Nitrobenzylidene)-2-(thiazol-2-ylimino)-4-thiazolidinone exhibiting anticonvulsant effect. Disclosure 12.10.2015.. 2016 Mar 10; UA 105330 (Ukraine).
8. Nektegayev I, Lesyk R. ChemInform Abstract: 3-Oxyaryl-2-thionethiazolidones-4 and Their Choleretic Activity. Scientia Pharmaceutica. 2010 Mar;67(10):227-30. https://doi.org/10.1002/chin.200010126
9. Boyd MR, Paull KD. Some practical considerations and applications of the national cancer institute in vitro anticancer drug discovery screen. Drug Development Research. 1995 Feb;34(2):91-109. https://doi.org/10.1002/ddr.430340203
10. Shoemaker RH. The NCI60 human tumour cell line anticancer drug screen. Nature Reviews Cancer. 2006 Oct;6(10):813-823. https://doi.org/10.1038/nrc1951
11. Paull KD, Shoemaker RH, Hodes L, Monks A, Scudiero DA, Rubinstein L, Plowman J, Boyd MR. Display and Analysis of Patterns of Differential Activity of Drugs Against Human Tumor Cell Lines: Development of Mean Graph and COMPARE Algorithm. JNCI Journal of the National Cancer Institute. 1989 Jul 19;81(14):1088-1092. https://doi.org/10.1093/jnci/81.14.1088
12. Havrylyuk D, Mosula L, Zimenkovsky B, Vasylenko O, Gzella A, Lesyk R. Synthesis and anticancer activity evaluation of 4-thiazolidinones containing benzothiazole moiety. European Journal of Medicinal Chemistry. 2010 Nov;45(11):5012-5021. https://doi.org/10.1016/j.ejmech.2010.08.008
13. Kaminskyy D, den Hartog GJ, Wojtyra M, Lelyukh M, Gzella A, Bast A, Lesyk R. Antifibrotic and anticancer action of 5-ene amino/iminothiazolidinones. European Journal of Medicinal Chemistry. 2016 Apr;112:180-195. https://doi.org/10.1016/j.ejmech.2016.02.011
14. Senkiv J, Finiuk N, Kaminskyy D, Havrylyuk D, Wojtyra M, Kril I, Gzella A, Stoika R, Lesyk R. 5-Ene-4-thiazolidinones induce apoptosis in mammalian leukemia cells. European Journal of Medicinal Chemistry. 2016 Jul;117:33-46. https://doi.org/10.1016/j.ejmech.2016.03.089
15. Kaminskyy D, Khyluk D, Vasylenko O, Zaprutko L, Lesyk R. A Facile Synthesis and Anticancer Activity Evaluation of Spiro[Thiazolidinone-Isatin] Conjugates. Scientia Pharmaceutica. 2011;79(4):763-777. https://doi.org/10.3797/scipharm.1109-14
16. Havrylyuk D, Zimenkovsky B, Vasylenko O, Gzella A, Lesyk R. Synthesis of New 4-Thiazolidinone-, Pyrazoline-, and Isatin-Based Conjugates with Promising Antitumor Activity. Journal of Medicinal Chemistry. 2012 Oct 9;55(20):8630-8641. https://doi.org/10.1021/jm300789g
17. Kaminskyy D, Zimenkovsky B, Lesyk R. Synthesis and in vitro anticancer activity of 2,4-azolidinedione-acetic acids derivatives. European Journal of Medicinal Chemistry. 2009 Sep;44(9):3627-3636. https://doi.org/10.1016/j.ejmech.2009.02.023
18. Havrylyuk D, Zimenkovsky B, Lesyk R. Synthesis and Anticancer Activity of Novel Nonfused Bicyclic Thiazolidinone Derivatives. Phosphorus, Sulfur, and Silicon and the Related Elements. 2009 Mar 10;184(3):638-650. https://doi.org/10.1080/10426500802247563
19. Subtel'na I, Atamanyuk D, Szymańska E, Kieć-Kononowicz K, Zimenkovsky B, Vasylenko O, Gzella A, Lesyk R. Synthesis of 5-arylidene-2-amino-4-azolones and evaluation of their anticancer activity. Bioorganic & Medicinal Chemistry. 2010 Jul;18(14):5090-5102. https://doi.org/10.1016/j.bmc.2010.05.073
20. Havrylyuk D, Kovach N, Zimenkovsky B, Vasylenko O, Lesyk R. Synthesis and Anticancer Activity of Isatin-Based Pyrazolines and Thiazolidines Conjugates. Archiv der Pharmazie. 2011 Jun 16;344(8):514-522. https://doi.org/10.1002/ardp.201100055
21. Avdieiev S, Gera L, Havrylyuk D, Hodges RS, Lesyk R, Ribrag V, Vassetzky Y, Kavsan V. Bradykinin antagonists and thiazolidinone derivatives as new potential anti-cancer compounds. Bioorganic & Medicinal Chemistry. 2014 Aug;22(15):3815-3823. https://doi.org/10.1016/j.bmc.2014.06.046
22. Kaminskyy D, Subtel'na I, Zimenkovsky B, Karpenko O, Gzella A, Lesyk R. Synthesis and Evaluation of Anticancer Activity of 5-Ylidene-4- Aminothiazol-2-(5H)-one Derivatives. Medicinal Chemistry. 2015 Jul 24;11(6):517-530. https://doi.org/10.2174/1573406411666150211112049
23. Szychowski KA, Leja ML, Kaminskyy DV, Binduga UE, Pinyazhko OR, Lesyk RB, Gmiński J. Study of novel anticancer 4-thiazolidinone derivatives. Chemico-Biological Interactions. 2017 Jan;262:46-56. https://doi.org/10.1016/j.cbi.2016.12.008
24. Szychowski KA, Leja ML, Kaminskyy DV, Kryshchyshyn AP, Binduga UE, Pinyazhko OR, Lesyk RB, Tobiasz J, Gmiński J. Anticancer properties of 4-thiazolidinone derivatives depend on peroxisome proliferator-activated receptor gamma (PPARγ). European Journal of Medicinal Chemistry. 2017 Dec;141:162-168. https://doi.org/10.1016/j.ejmech.2017.09.071
25. Szychowski KA, Kaminskyy DV, Leja ML, Kryshchyshyn AP, Lesyk RB, Tobiasz J, Wnuk M, Pomianek T, Gmiński J. Anticancer properties of 5Z-(4-fluorobenzylidene)-2-(4-hydroxyphenylamino)-thiazol-4-one. Scientific Reports. 2019 Jul 23;9(1). https://doi.org/10.1038/s41598-019-47177-6
26. Lozynskyi A, Golota S, Zimenkovsky B, Atamanyuk D, Gzella A, Lesyk R. Synthesis, anticancer and antiviral activities of novel thiopyrano[2,3-d]thiazole-6-carbaldehydes. Phosphorus, Sulfur, and Silicon and the Related Elements. 2016 Mar 30;191(9):1245-1249. https://doi.org/10.1080/10426507.2016.1166108

27. Kryshchyshyn AP, Atamanyuk DV, Kaminskyy DV, Grellier P, Lesyk RB. Investigation of anticancer and antiparasitic activity of thiopyrano[2,3-d]thiazoles bearing norbornane moiety. Biopolymers and Cell. 2017 Jun 30;33(3):183-205. https://doi.org/10.7124/bc.00094f
28. Atamanyuk D, Zimenkovsky B, Lesyk R. Synthesis and anticancer activity of novel thiopyrano[2,3-d]thiazole-based compounds containing norbornane moiety. Journal of Sulfur Chemistry. 2008 Apr;29(2):151-162. https://doi.org/10.1080/17415990801911723
29. Atamanyuk D. Synthesis and Biological Activity of New Thiopyrano[2,3-d]thiazoles Containing a Naphthoquinone Moiety. Scientia Pharmaceutica. 2013;81(2):423-436. https://doi.org/10.3797/scipharm.1301-13
30. Zelisko NI, Finiuk NS, Shvets VM, Medvid YO, Stoika RS, Lesyk RB. Screening of spiro-substituted thiopyrano[2,3-d]thiazoles for their cytotoxic action on tumor cells. Biopolymers and Cell. 2017 Aug 31;33(4):282-290. https://doi.org/10.7124/bc.00095a
31. Zimenkovsky B, Devinyak O, Havrylyuk D, Lesyk R. Analysis of antitumor activity of 4-thiazolidinone derivatives and their classification by probable mechanisms of action using mathematical statistics. Journal of Organic and Pharmaceutical Chemistry. 2011;9(3):64-71.
32. Zimenkovsky B, Devinyak O, Lesyk R. The study of the «structure – anticancer activity» relationship of 4-thiazolidinones using regression analysis and classification modeling. Journal of Organic and Pharmaceutical Chemistry. 2012;2:43-9.
33. Devinyak O, Lesyk R. Investigation of the relationship between the structure of 4-thiazolidinones and their antitumor activity by multidimensional adaptive regression splines. Journal of Organic and Pharmaceutical Chemistry. 2013;11(3):62-7.
34. Senkiv J, Finiuk N, Kaminskyy D, Havrylyuk D, Wojtyra M, Kril I, Gzella A, Stoika R, Lesyk R. 5-Ene-4-thiazolidinones induce apoptosis in mammalian leukemia cells. European Journal of Medicinal Chemistry. 2016 Jul;117:33-46. https://doi.org/10.1016/j.ejmech.2016.03.089
35. Chumak V, Fil M, Panchuk R, Zimenkovsky B, Havrylyuk D, Lesyk R, Stoika R. Study of antineoplastic action of novel isomeric derivatives of 4-thiazolidinone. Ukrainskiĭ biokhimicheskiĭ zhurnal. 2014;86:96-105.
36. Kobylinska LI, Boiko NM, Panchuk RR, Grytsyna II, Klyuchivska OY, Biletska LP, Lesyk RB, Zimenkovsky BS, Stoika RS. Putative anticancer potential of novel 4-thiazolidinone derivatives: cytotoxicity toward rat C6 glioma in vitro and correlation of general toxicity with the balance of free radical oxidation in rats. Croatian Medical Journal. 2016 Apr;57(2):151-163. https://doi.org/10.3325/cmj.2016.57.151
37. Havrylyuk D, Zimenkovsky B, Lesyk R, Stoika R, Lutsyk M, Panchuk R. 5-[2-Chloro-3-(4-nitrophenyl)-allylidene]-4-(3,5-diaryl-4,5-dihydropyrazol-1-yl)-5-H-thiazol-2-ones exhibiting antitumor activity in vitro and in vivo, and their solubilization method. Disclosure 21.08.2013. 2015 Jul 27; UA 109177 (Ukraine).
38. Zimenkovsky B, Devinyak O, Lesyk R. Modeling of a possible pharmacophore group for 4-thiazolidinones with the anticancer activity (Моделювання можливої фармакофорної групи 4-тіазолідинонів з протипухлинною активністю). Журнал органічної та фармацевтичної хімії. 2012;10:76-82.
39. Zimenkovsky BS, Lesyk RB, Devinyak OT, Havrylyuk DY. Method for antitumor activity predicting of 4-thiazolidinone derivatives. Disclosure 18.12.2012. 2013 May 27; UA 80435 (Ukraine).
40. Ouyang G, Cai X, Chen Z, Song B, Bhadury PS, Yang S, Jin L, Xue W, Hu D, Zeng S. Synthesis and Antiviral Activities of Pyrazole Derivatives Containing an Oxime Moiety. Journal of Agricultural and Food Chemistry. 2008 Nov 12;56(21):10160-10167. https://doi.org/10.1021/jf802489e
41. Havrylyuk D, Zimenkovsky B, Vasylenko O, Lesyk R. Synthesis and Anticancer and Antiviral Activities of New 2-Pyrazoline-Substituted 4-Thiazolidinones. Journal of Heterocyclic Chemistry. 2013 Feb;50(S1):E55-E62. https://doi.org/10.1002/jhet.1056
42. Zelisko NI, Demchuk I, Lesyk R. New thiopyrano[2,3-d][1,3]thiazole derivatives as potential antiviral agents. The Ukrainian Biochemical Journal. 2016 Jun 30;88(Special Issue):105-112. https://doi.org/10.15407/ubj88.si01.105
43. Havrylyuk D, Zimenkovsky B, Vasylenko O, Day CW, Smee DF, Grellier P, Lesyk R. Synthesis and biological activity evaluation of 5-pyrazoline substituted 4-thiazolidinones. European Journal of Medicinal Chemistry. 2013 Aug;66:228-237. https://doi.org/10.1016/j.ejmech.2013.05.044
44. Wojtyra MN, Lesyk RB, Zimenkovsky BS, Grellier P. (Z)-(5-[5-(3,5-diaryl-4,5-dihydropyrazol-1-yl-methylidene)-3-(pyridine-3-yl)-2-thioxothiazolidin-4-ones exhibiting antitrypanosomal action. Disclosure 18.04.2017. 2017 Oct 10; UA 119822 (Ukraine).
45. Holota S, Kryshchyshyn A, Derkach H, Trufin Y, Demchuk I, Gzella A, Grellier P, Lesyk R. Synthesis of 5-enamine-4-thiazolidinone derivatives with trypanocidal and anticancer activity. Bioorganic Chemistry. 2019 May;86:126-136. https://doi.org/10.1016/j.bioorg.2019.01.045
46. Kryshchyshyn A, Kaminskyy D, Karpenko O, Gzella A, Grellier P, Lesyk R. Thiazolidinone/thiazole based hybrids – New class of antitrypanosomal agents. European Journal of Medicinal Chemistry. 2019 Jul;174:292-308. https://doi.org/10.1016/j.ejmech.2019.04.052
47. Zelisko N, Karpenko O, Muzychenko V, Gzella A, Grellier P, Lesyk R. trans -Aconitic acid-based hetero -Diels-Alder reaction in the synthesis of thiopyrano[2,3- d][1,3]thiazole derivatives. Tetrahedron Letters. 2017 May;58(18):1751-1754. https://doi.org/10.1016/j.tetlet.2017.03.062
48. Zelisko N, Atamanyuk D, Vasylenko O, Grellier P, Lesyk R. Synthesis and antitrypanosomal activity of new 6,6,7-trisubstituted thiopyrano[2,3-d][1,3]thiazoles. Bioorganic & Medicinal Chemistry Letters. 2012 Dec;22(23):7071-7074. https://doi.org/10.1016/j.bmcl.2012.09.091

REVIEW PAPER

JMS Journal of Medical Science

Iodinated contrast media-induced hyperthyroidism

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ABSTRACT

Currently, iodinated contrast media (ICM) is widely used in radiology, therefore numerous patients are exposed to contrast administration during diagnostic and interventional procedures. ICM contains an amount of iodine well above the recommended dietary allowance, which can lead to thyroid dysfunction. Indeed, individuals that are highly susceptible to increased iodine intake are often patients with pre-existing thyroid disease. ICM-induced hyperthyroidism (IIH) is usually transient, however, it may present as clinically significant thyrotoxicosis. Although IIH has been investigated in multiple studies, there is still a lack of consensus regarding prophylactic therapy of IIH and no specific guidelines. This review aimed to summarise previous literature concerning the influence of ICM exposure on thyroid status and prophylactic therapy of IIH.

Introduction

Adequate iodine intake is essential for thyroid hormone synthesis, with insufficient, as well as excessive iodine intake leading to thyroid dysfunction. Individuals that are at risk of iodine-induced thyroid dysfunction are often patients with pre-existing thyroid disease, such as Graves' disease and multinodular goitre [1]. Iodine-induced hyperthyroidism, known as the Jod-Basedow phenomenon, may lead to severe health consequences and is especially important when considering elderly patients with comorbidities. The use of iodinated contrast media (ICM) during diagnostic and interventional procedures has increased considerably over the past years. ICM contains an amount of iodine which may result in thyroid dysfunction, such as hypo- and hyperthyroidism, both subclinical and overt [2]. ICM-induced hyperthyroidism (IIH) is usually transient, however, it may present as clinically significant thyrotoxicosis. Although IIH has been investigated, the prevalence has not been well established based on these studies and varies between 0% and 10%. Prophylactic therapy of IIH is still a matter of debate and the guidelines concerning this subject are not transparent. Until now, thiamazole and/or sodium perchlorate have been suggested for use as a prophylactic therapy of IIH [3]. This reviewed aimed to summarise the literature concerning the influence of ICM exposure on thyroid status and prophylactic therapy of IIH.

Thyroid function

Thyroid hormones, tetraiodothyronine (T4) and triiodothyronine (T3) are crucial for humans to maintain homeostasis, by regulating biochemical reactions, such as protein synthesis, enzymatic activity and are important for cell metabolism and immune response. They are also responsible for the correct development of the central nervous system, the musculoskeletal system and the lungs in the foetus and infants [4].

The production of thyroid hormones is dependent on the physiological function of the hypothalamus-pituitary-thyroid (HPT) axis and adequate release of thyrotropin-releasing hormone (TRH) and thyroid-stimulating hormone (TSH). TRH is synthesised in the periventricular nucleus of the hypothalamus, then released to the anterior pituitary gland, where it stimulates the biosynthesis and secretion of TSH in thyrotrope cells. TSH stimulates the production of T4 and T3 in the thyroid gland by binding to the thyroid-releasing hormone receptor (TSH-R) on the thyroid follicular cells. Thyroid hormone levels in the circulation determine the secretion of TRH and TSH by negative feedback [5].

The thyroid gland is composed of follicles, which consist of colloid surrounded by follicular cells. Thyroglobulin (TG) is a glycoprotein precursor of thyroid hormones, containing tyrosine residues within its structure. TG is produced by the endoplasmic reticulum of follicular cells of the thyroid and stored in the colloid [6]. Thyroid hormone synthesis is dependent on iodine metabolism, with iodide transported from the circulation into the thyroid cells by Na+/I- symporter located in thyroid epithelial cells and further iodide oxidised to iodine by the enzyme thyroid peroxidase. Oxidation leads to simultaneous iodination of tyrosyl residues in TG and formation of 3-monoiodotyrosine (MIT) and 3,5-diiodotyrosine (DIT). Afterwards, thyroid peroxidase catalyses coupling of MIT with DIT or two DITs, producing T3 and T4, respectively. Iodinated TG is then stored in the thyroid follicles. When thyroid hormone levels in the circulation decrease, internalisation of TG by follicular cells and digestion by lysosomal enzymes occurs, followed by secretion of free T4 and free T3 [7,8]. T4 and T3 then attach to thyroid hormone-binding proteins synthesised in the liver, including thyroxine-binding globulin (TBG), transthyretin (TTR), and albumin. Within the target cells, T4 is deiodinated to T3, which presents the greatest activity.

Iodine

The recommended dietary allowance (RDA) for iodine intake suggested by the World Health Organization is 90 µg for preschool children, 120 µg for schoolchildren, 150 µg for adolescents (above 12 years) and adults, and 250 µg for pregnant and lactating women [9]. Iodine is an element that occurs mostly as a salt and is present in many forms including inorganic iodine (I2), sodium and potassium salts, iodate, and iodide. It is widely present in food, added to salt as iodate and is also offered as a dietary supplement. Most iodine consumed by humans is reduced in the gastrointestinal tract to iodide and absorbed in the stomach and duodenum [10], then enters the circulation and is retrieved mostly by the thyroid gland and kidneys. It is concentrated in the thyroid in amounts adequate for hormone synthesis. An adult contains about 15–20 mg of iodine, 70–80% of which is stored in the thyroid, one-third in the form of thyroid hormone and two-thirds as precursors [11]. The iodine stored in thyroglobulin is removed from the tyrosine by a specific deiodinase and recycled within the thyroid. The iodine of T4 is returned to the serum after deiodination of T4 and may be included again in the cycle of iodine or excreted in the urine. The remaining iodine is excreted with median concentrations of 100–199 µg/L in children and adults, 150–249 µg/L in pregnant women and >100 µg/L in lactating women [9].

According to the WHO global scorecard of iodine nutrition in 2019, among countries with available data on iodine status, 135 presented with adequate, 25 insufficient and 14 with excessive iodine intake [12]. Iodine prophylaxis in Poland includes obligatory iodisation of household salt and neonate formula and additional supplementation for pregnant and breastfeeding women with 150–200 µg of iodine. In 1994, the Polish Council for Control of Iodine Deficiency Disorders (PCCIDD) defined iodine deficiency in Poland as moderate and slight in the coastal area. In 1997, the iodisation of table salt was re-introduced and Poland is currently within the European countries with optimal supplementation of iodine [13].

An iodine-deficient population is defined by a median urinary iodine concentration (UIC) below 100 µg/l for nonpregnant woman and children [14]. During iodine deficiency, TSH secretion rises when iodine intake decreases below 100 µg per day. Very low iodine intake may result in reduced thyroid hormone production, causing hypothyroidism, sometimes accompanied by goitre. Chronic iodine deficiency is associated with an increased risk of the follicular thyroid cancer [15]. Iodine deficiency is a dangerous state during pregnancy, as it can lead to miscarriage, stillbirth, neurodevelopmental deficits and growth abnormalities in the foetus [10]. Proper iodine intake prevents cretinism in the foetus, a disorder characterised by physical and neurological abnormalities such as intellectual disability, deaf-mutism and motor spasticity.

The tolerable upper intake level for iodine (UL) is the highest level of iodine consumption that is unlikely to cause adverse effects in most individuals. The value of UL has not been established and varies between 600 and 1100 µg/day. Gardner et al. and Paul et al. conducted studies that involved the supplemental intake of 500 and 1500 µg iodine per day, in addition to the usual iodine intake in the diet (200–300 µg/day). They showed that for those who were supplemented with 1500 µg of iodine per day, TSH concentrations increased significantly with a decrease in serum T4 concentration. It was also observed that the supplementation of 500 µg iodine daily caused no significant changes in the basal serum TSH or T4 levels [16,17]. Based on these results, the lowest-observed-adverse-effect-level (LOAEL) of iodine intake was assumed to be 1700 µg/day. The Institute of Medicine set UL at 1100 µg/day for adults by dividing the LOAEL by an uncertainty factor (UF), also known as the margin-of-safety, which was determined by the Institute of Medicine to be 1.5 [4]. The European Commission Scientific Committee on Food selected higher UF of 3 and set UL at 600 µg/day [18]. The Expert Group on Vitamins and Minerals and the Council for Responsible Nutrition established a guidance level for supplemental iodine intake of 500 µg/day [19,20]. The American Thyroid Association (ATA) caution against the ingestion of iodine and kelp supplements with the amount of iodine above 500 µg/day [21].

Amounts greater than UL may lead to thyroid dysfunction, such as thyroiditis, goitre, hypothyroidism and hyperthyroidism, both subclinical and overt, and sensitivity reactions [4]. In the state of excess iodine, Wolff-Chaikoff effect occurs, resulting in the discontinuation of the production and release of thyroid hormones. This effect is mostly transient, however, failing to escape from the acute Wolff-Chaikoff effect may lead to iodine-induced hypothyroidism. Another effect caused by excess iodine intake is the Jod-Basedow phenomenon (iodine-induced hyperthyroidism), which occurs mostly in individuals with dysregulation of the thyroid follicular cell [22]. The most susceptible to the adverse effects of excess iodine are patients with autonomously functioning nodular goitre living in moderate to mild iodine-deficient areas, patients with Graves' disease, also in remission after treatment, Hashimoto's disease, history of partial thyroidectomy [21,23,24]. Iodine-induced hyperthyroidism has also been reported in a situation of excess iodine in euthyroid patients with nodular goitre in iodine sufficient areas [25].

ICM-induced thyroid dysfunction

ICM typically contains 13500 µg of free iodine and 15–60 g of bound iodine [2], a quantity greatly exceeding UL. Contrast media is widely used in radiology to increase the contrast between the tissues in the images. Iodine-based contrast agents are commonly used during computed tomography (CT) and interventional procedures. However, they are known to cause various adverse effects, leading to acute reactions such as allergy-like or hypersensitivity reactions or chemotoxic responses. Late adverse reactions include post-contrast acute kidney injury and thyroid dysfunction [26]. ICM can be classified as high, low and iso-osmolar, with high osmolar ICM ranging from 1400 to 2500 mOsm/kg, low osmolar from 290 to 702 mOsm/kg, and iso-osmolar of 290 mOsm/kg. ICM with an osmolality closer to serum osmolality (285–295 mOsm/kg) has a lower incidence of side effects but contain more particles in solution per iodine atom (iodine ratio). Nevertheless, both high and low osmolar ICM contain an amount of iodine well above the RDA. Low and iso-osmolar ICM are more frequently used because of the lower risk of renal adverse effects in patients with chronic kid-

ney disease [27,28]. The required amount of ICM varies between 50 and 100 mL for CT scan and up to 200 mL for invasive procedures [29], and iodine levels continue to be elevated for up to 1–2 months after ICM injection [30]. ICM routinely used in practice are presented in **Table 1**.

ICM exposure is associated with an increased risk of developing thyroid dysfunction. Indeed, IIH has been confirmed in susceptible individuals as well as in patients with intact thyroid [31]. In a group of patients without pre-existing thyroid disease, Rhee et al. observed a 2–3 times increased probability of developing hyperthyroidism after coronary angiography compared to the control group [2]. The incidence of IIH is greater in iodine-deficient areas (up to 1.7%) and low in iodine sufficient areas [32]. Although IIH has been confirmed by multiple studies, the prevalence of IIH remains unclear [30,33-40], with the prevalence of subclinical IIH varying from 0% to 9% and overt IIH ranges between 0% and 10% (**Table 2**). The effect of ICM on thyroid status is believed to be transient and monitoring of thyroid function before and after ICM administration is not generally recommended [41]. However, published data shows that exposure to ICM can lead to severe thyrotoxicosis resulting in thyroid storm, cardiogenic shock and cardiopulmonary arrest [42,43]. Some authors recommend that patients who present risk factors for IIH should be examined for thyroid dysfunction after ICM [21].

ICM administration may also lead to the development of ICM-induced hypothyroidism, which is usually transient. In 2 to 3 weeks after iodide withdrawal, thyroid hormone synthesis usually returns to normal but some patients may develop permanent hypothyroidism [44], hence, the monitoring of patients diagnosed with ICM-induced hypothyroidism is necessary.

Prophylactic therapy

Prophylactic therapy of IIH has not been sufficiently investigated in prospective studies, with only a few studies on small groups of patients performed. Thiamazole and/or sodium perchlorate are generally considered as prophylactic therapy of IIH, but specific guidelines are needed to establish the regimen of prophylaxis. Thiama-

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

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)	nonionic monomer	774	3.0	370
iohexol 300 (Omnipaque)	nonionic monomer	672	3.0	300
iomeprol 350 (Iomeron)	nonionic monomer	618	3.0	350
iohexol 240 (Omnipaque)	nonionic monomer	518	3.0	240
iodixanol 320 (Visipaque)	nonionic dimer	290	6.0	320

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

Table 2. Summary of the studies that investigated ICM-induced hyperthyroidism

Study	Study group (n)	Follow-up (weeks)	Subclinical Hyperthyroidism n (%)	Overt Hyperthyroidism n (%)
Jarvis et al. [37]	102	8	2 (2.0)	0
Conn et al. [40]	73	8	4 (5.4)	2 (2.7)
Bonelli et al. [39]	810	52	74 (9.1)	7 (0.8)
Hintze et al. [38]	788	12	27 (4.9)	3 (0.4)
Ozkan et al. [34]	101	8	7 (6.9)	0
Lee et al. [30]	49	4	4 (8.1)	1 (2.0)
Skórkowska-Telichowska et al. [33]	59	26	3 (5.0)	6 (10.1)
Koroscil et al. [35]	56	1	0	0
Kaneshige et al. [36]	22	26	0	0

Abbreviations: ICM: iodinated contrast media

zole blocks the production of thyroid hormones by inhibiting thyroid peroxidase in the thyroid gland, thus inhibiting the iodination of tyrosine residues in TG [45]. Sodium perchlorate prevents iodide from entering the thyroid by an effect on the Na+/I- symporter, therefore stopping the synthesis of T3 and T4 [46]. Nolte et al. [47] observed that prophylactic treatment with monotherapy with 20 mg/day thiamazole or 900 mg/day sodium perchlorate has a protective effect against iodine excess in patients with euthyroid autonomy. Nevertheless, despite therapy with thiamazole or sodium perchlorate, two cases of mild hyperthyroidism have been reported. Another study investigated a group of 60 euthyroid patients, of which 27 individuals received prophylactic treatment with 60 mg methimazole and 1 g perchlorate administrated one day before and on the day of ICM exposure. Three cases of mild hyperthyroidism were observed in the control group. One case of hyperthyroidism was reported in the group with prophylaxis, however, this patient had another ICM injection without premedication 2 weeks after the first ICM injection [48]. Fricke et al. studied a group of 19 patients undergoing coronary angiography with technetium thyroid uptake greater than 1%. Patients were administered with the prophylactic treatment of 900 mg of perchlorate for two weeks in monotherapy or combined with 20 to 60 mg thiamazole for 1–2 weeks. Two cases of mild thyrotoxicosis were reported [49].

ATA does not recommend routine prophylaxis with antithyroid drugs before ICM but suggests considering prophylaxis in patients at risk of developing IIH [23]. The European Society of Urogenital Radiology (ESUR) introduced a sample combination regimen for prophylaxis with 30 mg once daily thiamazole and 300 mg three times a day sodium perchlorate, from the day before ICM and for the next 14 and 8–14 days, respectively. Both ATA and ESUR advise against the use of ICM in patients with hyperthyroidism [3].

Summary

In conclusion, IIH is an underestimated clinical condition, which, if not treated, may lead to severe health consequences, especially in the elderly and patients with cardiovascular disorders. The prevalence of IIH remains unclear. Until now, the prophylactic treatment of IIH with thiamazole and/or perchlorate in various regimens has been proposed, although the effectiveness of these drugs is uncertain. Further prospective, randomised studies on representative groups of patients concerning the prophylactic therapy of IIH are crucial for the proper prevention of this thyroid dysfunction.

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References

1. Thomsen HS, Morcos SK, van der Molen AJ. Effect of iodinated contrast media on thyroid function in adults. European Radiology. 2004 May 1;14(5):902-907. https://doi.org/10.1007/s00330-004-2238-z
2. Rhee CM. Association Between Iodinated Contrast Media Exposure and Incident Hyperthyroidism and Hypothyroidism. Archives of Internal Medicine. 2012 Jan 23;172(2):153. https://doi.org/10.1001/archinternmed.2011.677
3. Thomsen HS. European Society of Urogenital Radiology (ESUR) guidelines on the safe use of iodinated contrast media. European Journal of Radiology. 2006 Dec;60(3):307-313. https://doi.org/10.1016/j.ejrad.2006.06.020
4. Institute of Medicine. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Washington (DC): National Academies Press; 2001. https://doi.org/10.17226/10026
5. Ortiga Carvalho TM, Chiamolera MI, Pazos Moura CC, Wondisford FE. Hypothalamus-Pituitary-Thyroid Axis. Compr Physiol. 2016;6(3):1387-428. https://doi.org/10.1002/cphy.c150027
6. Coscia F, Taler-Verčič A, Chang VT, Sinn L, O'Reilly FJ, Izoré T, Renko M, Berger I, Rappsilber J, Turk D, Löwe J. The structure of human thyroglobulin. Nature. 2020 Feb;578(7796):627-630. https://doi.org/10.1038/s41586-020-1995-4
7. Schimmel M. Thyroidal and Peripheral Production of Thyroid Hormones. Annals of Internal Medicine. 1977 Dec 1;87(6):760. https://doi.org/10.7326/0003-4819-87-6-760
8. Mansourian A. Metabolic Pathways of Tetraidothyronine and Triidothyronine Production by Thyroid Gland: A Review of Articles. Pakistan Journal of Biological Sciences. 2011 Jan 1;14(1):1-12. https://doi.org/10.3923/pjbs.2011.1.12
9. World Health Organization. Assessment of iodine deficiency disorders and monitoring their elimi-

nation: a guide for programme managers, 3rd ed. https://apps.who.int/iris/handle/10665/43781. Accessed 2020 June.
10. Zimmermann MB. Iodine Deficiency. Endocrine Reviews. 2009 Jun;30(4):376-408. https://doi.org/10.1210/er.2009-0011
11. Zimmermann MB, Jooste PL, Pandav CS. Iodine-deficiency disorders. The Lancet. 2008 Oct;372(9645):1251-1262. https://doi.org/10.1016/s0140-6736(08)61005-3
12. The Iodine Global Network. Global scorecard of iodine nutrition in 2019 in the general population based on school-age children (SAC). https://www.ign.org/cm_data/Global_Scorecard_2019_SAC.pdf. Accessed 2020 June.
13. Szybiński Z, Polish Council for Control of Iodine Deficiency Disorders. Work of the Polish Council for Control of Iodine Deficiency Disorders, and the model of iodine prophylaxis in Poland. Endokrynol Pol. 2012;63(2):156-60. PMID 22538756
14. Iodine status worldwide. WHO Global Database on Iodine Deficiency. 2004. https://apps.who.int/iris/bitstream/handle/10665/43010/9241592001.pdf?sequence=1. Accessed 2020 June.
15. Zimmermann MB, Galetti V. Iodine intake as a risk factor for thyroid cancer: a comprehensive review of animal and human studies. Thyroid Research. 2015 Jun 18;8(1). https://doi.org/10.1186/s13044-015-0020-8
16. Paul T, Meyers B, Witorsch R, Pino S, Chipkin S, Ingbar S, Braverman L. The effect of small increases in dietary iodine on thyroid function in euthyroid subjects. Metabolism. 1988 Feb;37(2):121-124. https://doi.org/10.1016/s0026-0495(98)90004-x
17. Gardner D, Centor R, Utiger R. Effects of Low Dose Oral Iodide Supplementation on Thyroid Function in Normal Men. Clinical Endocrinology. 1988 Mar;28(3):283-288. https://doi.org/10.1111/j.1365-2265.1988.tb01214.x
18. Scientific Committee on Food. Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Iodine (expressed on 26 September 2002). https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scf_out146_en.pdf. Accessed 2020 June.
19. Expert Group on Vitamins and Minerals. Safe Upper Levels for Vitamins and Minerals. May 2003. https://cot.food.gov.uk/sites/default/files/vitmin2003.pdf. Accessed 2020 June.
20. Council for Responsible Nutrition. Vitamin and Mineral Safety 3rd Edition. 2014. https://www.crnusa.org/sites/default/files/files/resources/CRN-Safety-Book-3rdEdition-2014-fullbook.pdf. Accessed 2020 June.
21. Leung AM, Avram AM, Brenner AV, Duntas LH, Ehrenkranz J, Hennessey JV, Lee SL, Pearce EN, Roman SA, Stagnaro-Green A, Sturgis EM, Sundaram K, Thomas MJ, Wexler JA. Potential Risks of Excess Iodine Ingestion and Exposure: Statement by the American Thyroid Association Public Health Committee. Thyroid. 2015 Feb;25(2):145-146. https://doi.org/10.1089/thy.2014.0331
22. Leung AM, Braverman LE. Consequences of excess iodine. Nature Reviews Endocrinology. 2013 Dec 17;10(3):136-142. https://doi.org/10.1038/nrendo.2013.251
23. Ross DS, Burch HB, Cooper DS, Greenlee MC, Laurberg P, Maia AL, Rivkees SA, Samuels M, Sosa JA, Stan MN, Walter MA. 2016 American Thyroid Association Guidelines for Diagnosis and Management of Hyperthyroidism and Other Causes of Thyrotoxicosis. Thyroid. 2016 Oct;26(10):1343-1421. https://doi.org/10.1089/thy.2016.0229
24. Biban BG, Lichiardopol C. Iodine Deficiency, Still a Global Problem?. Current Health Sciences Journal. 2017 Jul 31;(2):103-111. https://doi.org/10.12865/CHSJ.43.02.01
25. Vagenakis AG, Wang C, Burger A, Maloof F, Braverman LE, Ingbar SH. Iodide-Induced Thyrotoxicosis in Boston. New England Journal of Medicine. 1972 Sep 14;287(11):523-527. https://doi.org/10.1056/nejm197209142871101
26. ESUR Contrast Media Safety Committee. ESUR Guidlines on Contrast Media. http://www.esur.org/guidelines/en/index.php#a. Accessed 2020 June.
27. Thomsen H, Bellin M, Jakobsen J, Webb J. Contrast Media Classification and Terminology. In: Thomsen H, Webb JAW, eds. Contrast Media Safety Issues and ESUR Guidelines. Berlin, Heidelberg: Springer Berlin Heidelberg; 2014:3-11.
28. Isola S, Furci F, Gangemi S. The involvement of osmolarity in the safety of contrast media. Clinical and Molecular Allergy. 2018 Sep 3;16(1). https://doi.org/10.1186/s12948-018-0097-4
29. Mujtaba F, Saghir T, Sial J, Nasseb K, Karim M. Evaluation of Contrast Amount in Patients Undergoing Cardiac Catheterization for Diagnostic and Therapeutic Procedures. EC CARDIOLOGY. 2019;6(2):102-8.
30. Lee SY, Chang DL, He X, Pearce EN, Braverman LE, Leung AM. Urinary Iodine Excretion and Serum Thyroid Function in Adults After Iodinated Contrast Administration. Thyroid. 2015 May;25(5):471-477. https://doi.org/10.1089/thy.2015.0024
31. Fradkin JE, Wolff J. Iodide-Induced Thyrotoxicosis. Medicine. 1983 Jan;62(1):1-20. https://doi.org/10.1097/00005792-198301000-00001
32. Roti E, Uberti ED. Iodine Excess and Hyperthyroidism. Thyroid. 2001 May;11(5):493-500. https://doi.org/10.1089/105072501300176453
33. Skórkowska-Telichowska K, Kosińska J, Szymczak R, Tuchendler D, Tuchendler R, Telichowski A, Szajn G, Bolanowski M. Comparison and assessment of thyroid morphology and function in inhabitants of Lower Silesia before and after administration of a single dose of iodine-containing contrast agent during cardiac intervention procedure. Endokrynol Pol. 2012;63(4):294-9.
34. Ozkan S, Oysu AS, Kayatas K, Demirtunç R, Eren M, Uslu H, Altuntas Y. Thyroid functions after contrast agent administration for coronary angiography: a prospective observational study in euthyroid patients. Anadolu Kardiyoloji Dergisi/The Anatolian Journal of Cardiology. 2013 Apr 24;. https://doi.org/10.5152/akd.2013.134

35. Koroscil, MD, PhD TM, Pelletier, MD PR, Slauson, MD JW, Hennessey, MD J. Short-term effects of coronary angiographic contrast agents on thyroid function. Endocrine Practice. 1997 Jul;3(4):219-221. https://doi.org/10.4158/ep.3.4.219
36. Kaneshige T, Arata N, Harada S, Ohashi T, Sato S, Umehara N, Saito T, Saito H, Murashima A, Sago H. Changes in Serum Iodine Concentration, Urinary Iodine Excretion and Thyroid Function After Hysterosalpingography Using an Oil-Soluble Iodinated Contrast Medium (Lipiodol). The Journal of Clinical Endocrinology & Metabolism. 2015 Mar;100(3):E469-E472. https://doi.org/10.1210/jc.2014-2731
37. Jarvis C, Simcox K, Tamatea JAU, McAnulty K, Meyer-Rochow GY, Conaglen JV, Elston MS. A low incidence of iodine-induced hyperthyroidism following administration of iodinated contrast in an iodine-deficient region. Clinical Endocrinology. 2015 Jun 19;84(4):558-563. https://doi.org/10.1111/cen.12818
38. Hintze G, Blombach O, Fink H, Burkhardt U, Kobberling J. Risk of iodine-induced thyrotoxicosis after coronary angiography: an investigation in 788 unselected subjects. European Journal of Endocrinology. 1999 Mar 1;:264-267. https://doi.org/10.1530/eje.0.1400264
39. Bonelli N, Rossetto R, Castagno D, Anselmino M, Vignolo F, Parasiliti Caprino M, Gaita F, Ghigo E, Garberoglio R, Grimaldi R, Maccario M. Hyperthyroidism in patients with ischaemic heart disease after iodine load induced by coronary angiography: Long-term follow-up and influence of baseline thyroid functional status. Clinical Endocrinology. 2017 Nov 20;88(2):272-278. https://doi.org/10.1111/cen.13494
40. Conn JJ, Sebastian MJ, Deam D, Tam M, Martin F. A Prospective Study of the Effect of Nonionic Contrast Media on Thyroid Function. Thyroid. 1996 Apr;6(2):107-110. https://doi.org/10.1089/thy.1996.6.107
41. Windecker S, Kolh P, Alfonso F, Collet J, Cremer J, Falk V, Filippatos G, Hamm C, Head SJ, Jüni P, Kappetein AP, Kastrati A, Knuuti J, Landmesser U, Laufer G, Neumann F, Richter DJ, Schauerte P, Sousa Uva M, Stefanini GG, Taggart DP, Torracca L, Valgimigli M, Wijns W, Witkowski A. 2014 ESC/EACTS Guidelines on myocardial revascularization. EuroIntervention. 2015 Jan;10(9):1024-1094. https://doi.org/10.4244/eijy14m09_01
42. Alkhuja S, Pyram R, Odeyemi O. In the eye of the storm: Iodinated contrast medium induced thyroid storm presenting as cardiopulmonary arrest. Heart & Lung. 2013 Jul;42(4):267-269. https://doi.org/10.1016/j.hrtlng.2013.04.002
43. Brundridge W, Perkins J. Iodinated Contrast Administration Resulting in Cardiogenic Shock in Patient with Uncontrolled Graves Disease. The Journal of Emergency Medicine. 2017 Dec;53(6):e125-e128. https://doi.org/10.1016/j.jemermed.2017.08.096
44. Markou K, Georgopoulos N, Kyriazopoulou V, Vagenakis A. Iodine-Induced Hypothyroidism. Thyroid. 2001 May;11(5):501-510. https://doi.org/10.1089/105072501300176462
45. Cooper DS. Antithyroid Drugs. New England Journal of Medicine. 2005 Mar 3;352(9):905-917. https://doi.org/10.1056/nejmra042972
46. Wolff J. Perchlorate and the thyroid gland. Pharmacol Rev. 1998;50(1):89-105.
47. Nolte W, Müller R, Siggelkow H, Emrich D, Hüfner M. Prophylactic application of thyrostatic drugs during excessive iodine exposure in euthyroid patients with thyroid autonomy: a randomized study. European Journal of Endocrinology. 1996 Mar;134(3):337-341. https://doi.org/10.1530/eje.0.1340337
48. Fritzsche H, Benzer W, Furlan W, Hammerle D, Langsteger W, Weiss P. Zur Vorbeugung jodinduzierter Hyperthyreosen nach Koronarangiographie [Prevention of iodine-induced hyperthyroidism after coronary angiography]. Acta Med Austriaca. 1-2;1993(20):13-7. PMID 8386417
49. Fricke E, Fricke H, Esdorn E, Kammeier A, Lindner O, Kleesiek K, Horstkotte D, Burchert W. Scintigraphy for Risk Stratification of Iodine-Induced Thyrotoxicosis in Patients Receiving Contrast Agent for Coronary Angiography: A Prospective Study of Patients with Low Thyrotropin. The Journal of Clinical Endocrinology & Metabolism. 2004 Dec;89(12):6092-6096. https://doi.org/10.1210/jc.2004-0728

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Endoscopic Treatment of Pancreatic Fluid Collections

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ABSTRACT

Acute pancreatitis is frequently complicated by pancreatic fluid collections (PFCs), which usually resolve spontaneously but some can mature forming large cysts filled with fluid or necrotic debris. Historically, they have been surgically removed but with the advancement of endoscopic procedures, endoscopic drainage has emerged as a safe first-line treatment of PFCs. Furthermore, the development of plastic stents and lumen apposing metal stents (LAMS) has replaced not only open surgery but also the percutaneous drainage due to fewer adverse events. In particular, the LAMS has gained favour recently as large meta-analysis suggested their advantages over plastic stents in the treatment of PFCs, however, data regarding their use in the drainage of PFCs are still scarce.

Introduction

Pancreatic fluid collections (PFCs) are common complications of acute pancreatitis n over 40% of patients [1]. evised Atlanta criteria categorie PFCs as acute (<4 weeks after episode of pancreatitis) or chronic (>4 weeks after episode of pancreatitis), which are further subdivided by the presence or absence of necrosis in the fluid collection. acute PFCs are acute peripancreatic fluid collections (APFCs) that do not have a defined wall and are reabsorbed spontaneously within several weeks. The remainder form acute necrotic collections (ANCs) consist of a combination of necrotic tissue and variable amount of fluid. ifferentiation between the two is difficult sequential imaging is often required. These PFCs can mature and form a capsule leading to creation of pancreatic pseudocysts (PP) and walled-off pancreonecrosis (WOPN) respectively [2]. Traditionally, large PP, WOPN or infected necroti pancreatitis can be treated with open necrosctomy with a recent tendency towards step up surgical approach based on percutaneous or endoscopic drainage. These minimally invasive procedures are associated with decreased mortality, multiorgan failures, and long term pancreatic endocrine and exocrine insufficiency [3,4]. Studies also attempted to compare the outcomes of percutaneous and endoscopic drainagedifference

in major complications or mortality between the two methods demonstrated, however, the percutaneous approach was complicated with an increased inflammatory respone, higher rate of pancreatic fistulas and longer hospital stay [5,6]. With furher advances in endoscopic procedures and the development of plastic stents (PS) and more recently, lumen aposing metal stents (LAMS), endoscopic drainage has become widely regarded as a safe first-line therapy for patient with necrotic or infected PFC, symptomatic PP that are anatomically amenable to drainage.

PS were the first utili in transmural endoscopic drainage of PFCs. Initially gastroscopic evaluation performed to identify PFCs by extrinsic bulging compressing the gastric lumen. Afterwards multiple PS could be positioned transmuraly with placement with endoscopic and fluoroscopic guidance. This approach has evolved with the development of endoscopic ultrasound (EUS) as the PFCs could be directly visuali allowing for more precise stent placement, thereby associated with decreased number of complications. PS have been proven effective for drainage of PP with complete resolution of the PFCs in 8293% of cases with cyst reoccurrence [7-10]. Nevertheless, EUS assisted PFCs drainage with the use of PS is associated with multiple complications including acute bleeding episodes, stent occlusions or migration, infection and perforation that occur in 240% of patients [11]. Although intervention with PS have proven to be effective in the treatment of fluidfilled cysts, WOPN cavities with more solid debris have led to increased risk of stent obstruction due to small diameter of PS [12].

Monumental advances to endoscopic intervention of PFCs arrived with the development of LAMS. These stents are similarly placed across luminal structures to create gastro-pancreatic connection. LAMS are tubularshaped biflanged which allows proper anchoragedecreas the risk of migration (**Figure 1**). Placement of LAMS has allowed for more efficient drainage of PFCs due to the larger diameter size while allowing direct interrogation of the cyst cavity through the stent and subsequent intervention such us necrosectomy. This is usually performed in WOPN with large necrotic component, in infected necroses or infected fluid collections [13].

There are multiple LAMS currently available on the marketAxios (Boston Scientific, USA)

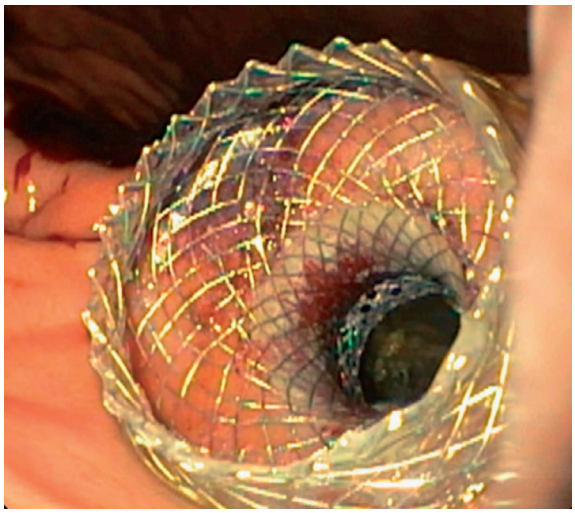


Figure 1. Endoscopic view of LAMS

Hanaro (M.I Tech, South Korea), Nagi and Spaxus (Taewong Medical, South Korea), Aix PPS (Leufen Medical, Germany), that vary in length (from 5 mm to 30 mm) and diameter (from 8 mm to 16 mm) (**Figure 2**). Initial studies have not shown benefits of LAMS over PS in PP and LAMS were believed to have more indications in the setting of WOPN, yet many studies revealed contradict results. However, large metaanalysis of Yoon (PP 250 patients, WOPN 555 patients) provided data the more frequent use of LAMS as they demonstrated higher technical and clinical success rate with dverse events after LAMS placement compared to PS.

Recent large meta-analysis of 14 studies from 2012 to 2016 that included 812 patients (608 WOPN, 204 PP) demonstrated high technical and clinical success rate of LAMS in the treatment of PFCs. LAMS were successfully placed in 98.9% and resolution of patient symptoms with at least 50% size reduction of PFCs was accomplished in over 90% of patients with WOPN. In the treatment of PP the technical and clinical success was even higher, respectively in 97% and 98% of patients. Unfortunately, 10.1% of patients developed complicationsoccurred early and included infections (3.6%), bleeding (3.3%) or stent migration (1.9%). Fortunately, major events as perforations occurred in 0.6% of patients [14]. Our data also demonstrated promising outcomes, as none of the 43 patients with PFCs treated with LAMS developed any major complications and adverse events were mostly limited to minor acute bleeding episodes resolving after cauteri (4.7%) or stent migrations (7.0%) [15].

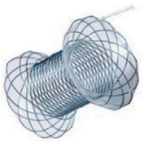
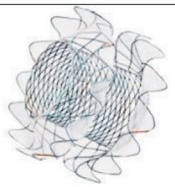
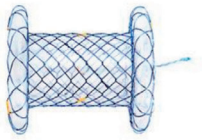
Nagi (Taewong Medical, South Korea)		Axios (Boston Scientific, USA)	
Spaxus (Taewong Medical, South Korea)		Plumber (M.I Tech, South Korea)	
Aix PPS (Leufen Medical, Germany)			

Figure 2. Currently available LAMS

The newest modification, adopted by advanced endoscopists in 2015, led to integration of LAMS with electrocautery enhanced delivery system that enables advancement of the stent using cautery instead of prior dilation. This has resulted in reduced risk of malposition, leakage and is very cost [16].

In conclusion, with the advancements of endoscopic procedures, this minimally invasive approach using PS and LAMS becme mainstay of treatment of PFCs. The endoscopic approach associated with decreased mortality and morbidity over a surgical approachadverse events tha percutaneous drainage. The development of LAMS approach as recent data suggests advantages over PS in the treatment of PFCs but further randomi controlled trials are needed.

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

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References

1. Cui ML, Kim KH, Kim HG, Han J, Kim H, Cho KB, Jung MK, Cho CM, Kim TN. Incidence, Risk Factors and Clinical Course of Pancreatic Fluid Collections in Acute Pancreatitis. Digestive Diseases and Sciences. 2013 Dec 11;59(5):1055-1062. https://doi.org/10.1007/s10620-013-2967-4
2. Banks PA, Bollen TL, Dervenis C, Gooszen HG, Johnson CD, Sarr MG, Tsiotos GG, Vege SS. Classification of acute pancreatitis—2012: revision of the Atlanta classification and definitions by international consensus. Gut. 2012 Oct 25;62(1):102-111. https://doi.org/10.1136/gutjnl-2012-302779
3. van Santvoort HC, Besselink MG, Bakker OJ, Hofker HS, Boermeester MA, Dejong CH, van Goor H, Schaapherder AF, van Eijck CH, Bollen TL, van Ramshorst B, Nieuwenhuijs VB, Timmer R, Laméris JS, Kruyt PM, Manusama ER, van der Harst E, van der Schelling GP, Karsten T, Hesselink EJ, van Laarhoven CJ, Rosman C, Bosscha K, de Wit RJ, Houdijk AP, van Leeuwen MS, Buskens E, Gooszen HG. A Step-up Approach or Open Necrosectomy for Necrotizing Pancreatitis. New England Journal of Medicine. 2010 Apr 22;362(16):1491-1502. https://doi.org/10.1056/nejmoa0908821
4. Hollemans RA, Bakker OJ, Boermeester MA, Bollen TL, Bosscha K, Bruno MJ, Buskens E, Dejong CH, van Duijvendijk P, van Eijck CH, Fockens P, van Goor H, van Grevenstein WM, van der Harst E, Heisterkamp J, Hesselink EJ, Hofker S, Houdijk AP, Karsten T, Kruyt PM, van Laarhoven CJ, Laméris JS, van Leeuwen MS, Manusama ER, Molenaar IQ, Nieuwenhuijs VB, van Ramshorst B, Roos D, Rosman C, Schaapherder AF, van der Schelling GP, Timmer R, Verdonk RC, de Wit RJ, Gooszen HG, Besselink MG, van Santvoort HC. Superiority of Step-up Approach vs Open Necrosectomy in Long-term Follow-up of Patients With Necrotizing Pancreatitis. Gastroenterology. 2019 Mar;156(4):1016-1026. https://doi.org/10.1053/j.gastro.2018.10.045
5. van Brunschot S, van Grinsven J, van Santvoort HC, Bakker OJ, Besselink MG, Boermeester MA, Bollen TL, Bosscha K, Bouwense SA, Bruno MJ, Cappendijk VC, Consten EC, Dejong CH, van Eijck CH, Erkelens WG, van Goor H, van Grevenstein WMU, Haveman J, Hofker SH, Jansen JM, Laméris JS, van

Lienden KP, Meijssen MA, Mulder CJ, Nieuwenhuijs VB, Poley J, Quispel R, de Ridder RJ, Römkens TE, Scheepers JJ, Schepers NJ, Schwartz MP, Seerden T, Spanier BWM, Straathof JWA, Strijker M, Timmer R, Venneman NG, Vleggaar FP, Voermans RP, Witteman BJ, Gooszen HG, Dijkgraaf MG, Fockens P, Manusama ER, Hadithi M, Rosman C, Schaapherder AF, Schoon EJ. Endoscopic or surgical step-up approach for infected necrotising pancreatitis: a multicentre randomised trial. The Lancet. 2018 Jan;391(10115):51-58. https://doi.org/10.1016/s0140-6736(17)32404-2

6. Bakker OJ, van Santvoort HC, van Brunschot S, Geskus RB, Besselink MG, Bollen TL, van Eijck CH, Fockens P, Hazebroek EJ, Nijmeijer RM, Poley J, van Ramshorst B, Vleggaar FP, Boermeester MA, Gooszen HG, Weusten BL, Timmer R, Dutch Pancreatitis Study Group. Endoscopic Transgastric vs Surgical Necrosectomy for Infected Necrotizing Pancreatitis. JAMA. 2012 Mar 14;307(10):1053. https://doi.org/10.1001/jama.2012.276
7. Kahaleh M, Shami V, Conaway M, Tokar J, Rockoff T, De La Rue S, de Lange E, Bassignani M, Gay S, Adams R, Yeaton P. Endoscopic Ultrasound Drainage of Pancreatic Pseudocyst: A Prospective Comparison with Conventional Endoscopic Drainage. Endoscopy. 2006 Apr;38(4):355-359. https://doi.org/10.1055/s-2006-925249
8. Cremer M, Deviere J, Engelholm L. Endoscopic management of cysts and pseudocysts in chronic pancreatitis: long-term follow-up after 7 years of experience. Gastrointestinal Endoscopy. 1989 Jan;35(1):1-9. https://doi.org/10.1016/s0016-5107(89)72677-8
9. Antillon MR, Shah RJ, Stiegmann G, Chen YK. Single-step EUS-guided transmural drainage of simple and complicated pancreatic pseudocysts. Gastrointestinal Endoscopy. 2006 May;63(6):797-803. https://doi.org/10.1016/j.gie.2005.10.025
10. Binmoeller KF, Seifert H, Walter A, Soehendra N. Transpapillary and transmural drainage of pancreatic pseudocysts. Gastrointestinal Endoscopy. 1995 Sep;42(3):219-224. https://doi.org/10.1016/s0016-5107(95)70095-1
11. Kahaleh M, Saumoy M, Arvanitakis M. Pancreatic fluid collections and necrosectomy with plastic stents versus lumen-apposing stents. Endoscopic Ultrasound. 2017;6(9):132. https://doi.org/10.4103/eus.eus_80_17
12. Chen Y, Yang J, Friedland S, Holmes I, Law R, Hosmer A, Stevens T, Franco M, Jang S, Pawa R, Mathur N, Sejpal D, Inamdar S, Trindade A, Nieto J, Berzin T, Sawhney M, DeSimone M, DiMaio C, Kumta N, Gupta S, Yachimski P, Anderloni A, Baron T, James T, Jamil L, Ona M, Lo S, Gaddam S, Dollhopf M, Bukhari M, Moran R, Gutierrez O, Sanaei O, Fayad L, Ngamruengphong S, Kumbhari V, Singh V, Repici A, Khashab M. Lumen apposing metal stents are superior to plastic stents in pancreatic walled-off necrosis: a large international multicenter study. Endoscopy International Open. 2019 Feb 28;7(3):E347-E354. https://doi.org/10.1055/a-0828-7630
13. Yan L, Dargan A, Nieto J, Shariaha RZ, Binmoeller KF, Adler DG, DeSimone M, Berzin T, Swahney M, Draganov PV, Yang DJ, Diehl DL, Wang L, Ghulab A, Butt N, Siddiqui AA. Direct endoscopic necrosectomy at the time of transmural stent placement results in earlier resolution of complex walled-off pancreatic necrosis: Results from a large multicenter United States trial. Endoscopic Ultrasound. 2019;8(3):172. https://doi.org/10.4103/eus.eus_108_17
14. Han D, Inamdar S, Lee CW, Miller LS, Trindade AJ, Sejpal DV. Lumen Apposing Metal Stents (LAMSs) for Drainage of Pancreatic and Gallbladder Collections. Journal of Clinical Gastroenterology. 2018 Oct;52(9):835-844. https://doi.org/10.1097/mcg.0000000000000934
15. Kloska M, Hrad V, Aggarwal S, Shah S, Zator Z, Shah H. Endoscopic Ultrasound-Guided Drainage of Pancreatic Fluid Collections Using AXIOS Electrocautery Enhanced Delivery System a Retrospective Experience at a Large Quaternary Care Center. American Journal of Gastroenterology. 2019 Oct;114:S544. https://doi.org/10.14309/01.ajg.0000593272.21427.04
16. Venkatachalapathy S, Makin A, Pereira S, Johnson G, Bekkali N, Penman I, Oppong K, Nayar M, Carroll N, Godfrey E, Ryan B, Parihar V, McKay C, Huggett M. OC-073 The First Multicentre Experience from The UK and Ireland of the Use of the Hot Axios System for Transluminal Drainage of Pancreatic Fluid Collections. Gut. 2016 Jun;65(Suppl 1):A44.1-A44. https://doi.org/10.1136/gutjnl-2016-312388.72

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Towards effective collaboration of physicians and pharmacists for the care of older people (including COVID-19 perspective)

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ABSTRACT

Collaboration between physicians and pharmacists is essential for proper and effective medical care. Medicine is a multifaceted discipline where success is judged by the outcomes of the patient's wellbeing. Incorporating interdisciplinary education within healthcare enhances the cooperation between future medical professionals. In this comprehensive review, we present the current steps that have been implemented to apply interdisciplinary care as well as interprofessional cooperation possibilities during the COVID-19 pandemic.

Introduction

Polypharmacy remains a pivotal challenge in geriatric medicine [1]. Older individuals experience drug-related problems (DRPs) with common side effects limiting the desired treatment outcomes and efficacy. The modern therapeutic model regarding the care of such issues in geriatric patients undoubtedly demands a different approach, with the potential of an interdisciplinary avenue. The concept of close cooperation between physicians and clinically-oriented pharmacists has already flourished in many countries such as Canada, the Netherlands and the United Kingdom [2-4]. The collaboration between physician and pharmacist may have great value in these difficult times as a result of the COVID-19 pandemic. Due to a lack of scientifically-based treatments for COVID-19 infection, the complications of potential adverse effects, including polypharmacy, becomes more important. This is especially evident in older individuals, which have

shown infection susceptibility for the COVID-19 virus. Implementation of such collaboration can assure better control of potential disease and drug interactions as well as the consistent implementation of safeguards for pharmacotherapy and disease persistence. This is of great importance, especially during a pandemic as polypathology, if not controlled, can be a predisposing factor for the life-threatening course of COVID-19. This article presents a literature review of the potential implementation of the physician/pharmacist cooperation, with an emphasis on the COVID-19 pandemic and implementation potential for enhancing patient wellbeing.

Collaboration around the World

To understand the true nature of the physician/pharmacist interaction concerning polypharmacy, and its impacts on the quality of care in older patients, a literature review was performed using the PubMed database to identify relevant articles published in the last 5 years (2015–2019). The following keywords were generated for the search: pharmacist(s), general practitioner(s), primary health care, community pharmacy service(s), polypharmacy, older adult(s), pharmacist and physician cooperation. After segregating preliminary results, 47 articles were identified, of which six were designated for the final analysis. The following article exclusion criteria were used: lack of substantial results depicting collaboration between physicians and pharmacists, lack of intervention concerning the improvement of care, and shortage of visible cooperation between physicians and pharmacists. Favoured articles had to meet the following selection conditions: intervention which relates to polypharmacy among older patients and description of cooperation actions undertaken by physicians and pharmacists. Each of the chosen articles develops on the importance of collaborative work with an emphasis on improved care in elderly patients with polypharmacy. The literature provides insight and importance for interprofessional cooperation and its potential for implementation in therapeutic models, which could provide elderly patients with better care and wellbeing. A more detailed summary is presented in **Tables 1** and **2**. For a better understanding of possible ways of interprofessional cooperation and potentially beneficial outcomes regarding pharmacotherapy, see **Figure 1**.

The Significance of Potential Cooperation During the COVID-19 Pandemic

It has been noted that the discontinuity of treatment caused by the decreased accessibility of conventional healthcare services during a pandemic can promote deterioration of chronic diseases among elderly patients. Subsequently, this results in the need for emergency healthcare, thereby placing additional strain on the healthcare system [11]. According to Zheng et al., pharmacists should provide chronic disease treatment management at the counter, pay attention to medication adherence and ensure safe medication use [12]. The emergence of the pharmacist, as a partner to the physician in treatment counselling, has been noted during the coronavirus (COVID-19) pandemic [12].

Indeed, pharmacists have proven to be essential partners of physicians during the COVID-19 outbreak, with their input not only enhancing the essential care of patients but also alleviating the burden placed on the healthcare system. In response to the COVID-19 pandemic, Aruru and colleagues presented an essential framework for the implementation of pharmacists into public healthcare services [13]. Moreover, a collaboration between physicians and pharmacists during this outbreak may be crucial in the management of medication and treatment protocols of elderly patients. Medications administered in the treatment of COVID-19 pneumonia vary from potentially beneficial to those whose risks outweigh their potential medicinal benefits [14]. Thus, the potential toxicity of multiple drug schemes used in treating coronavirus severe acute respiratory syndrome may demand additional pharmacist input to ensure their safety. This is especially evident in the hectic healthcare environment as a result of the pandemic. Furthermore, consultations between these two professions have the potential to enhance the treatment in individual cases of COVID-19. It is worth emphasising that elderly patients who are more likely to present fulminant symptoms of COVID-19 may

Table 1. Studies concerning interprofessional collaboration between medical doctors and pharmacists from the last 5 years (2015–2019) obtained from the PubMed database

Study	Sample Size (Inclusion/exclusion criteria Included)	Objective/Methodology	Results
De Bock L et al. [5] (interventional study)	52 out of 261 participants were involved in the study (20%), the other 80% were excluded based on the following exclusion criteria: – age (<70 years), – polypharmacy (<5 drugs taken at home), – admission to the geriatric ward less than 3 months ago, – cognitive impairment, – absence of clinical pharmacist/no consent	– to evaluate the successes and barriers of the implementation of a pharmacist-led full medication review process in the geriatric ward; – extended medication reconciliation at the time of admission and discharge; – a medication review by the clinical pharmacist using appropriate medication therapy screening tools (STOPP/START and GheOP^{3}S)	– 122 discrepancies detected at the admission; – 254 potentially inappropriate medications detected; – 192 therapeutic recommendations were issued, of which, 13% were fully accepted and 6% required an adjustment (e.g., different dose, different alternative drug) – Medication Appropriateness Index (MAI) improved from 75% to 88.2%
Köberlein-Neu J et al. [6] (cluster-randomised trial)	162 patients consented in writing to participate, of which, 142 patients were included in the intention-to-treat analysis Inclusion criteria: – Age ≥ 65 years, – a minimum of 3 chronic disorders affecting 2 different organ systems, – at least 1 cardiovascular disease, – at least 1 visit to the primary care physician in each of the preceding 3-month intervals, – 5 or more long-term drug treatments (>3 months) with systemic effects, – ability to complete questionnaires, with assistance if required, Exclusion criteria: – Life expectancy of less than 12 months (assessed by the treating primary care physician)	– to evaluate the efficacy of interprofessional medication management for multi-morbid patients; – medication management consulted with the pharmacist and care provided by the Pflege- und Wohnberatung (PuW, home-care specialists) using a case management concept according to the German Society for Care and Case Management (Deutsche Gesellschaft für Care und Case Management, DGCC) after obtaining recommendations	– significant decrease in the MAI score in the intervention phase (22.27, 95% CI 19.00; 25.54) in comparison to the control phase (29.21, 95% CI 26.09; 32.33)
Nachtigall A et al. [7] (prospective, controlled trial)	411 patients recruited (intervention group: n=209, control group: n=202) and allocated according to the ward they were treated on (ward A - intervention group, ward B - control group); Inclusion criteria: – age (≥70 years), – polypharmacy (≥ 5 drugs), – written informed consent Exclusion criteria: – patients with an estimated life expectancy of less than 1 week, – cognitive impairment, – previous participation in this study during the last 3 months in the same hospital	– medication analysis in search of DRPs; – priority classified recommendations were prepared for treating physicians to reduce DRPs and improve MAI	– the percentage of patients with DRPs reduced from 86.6% to 56.0% in the intervention group, from 76.7% to 76.2% in the control group ($p < 0.001$); – MAI score reduced by 56% in the intervention group and 0.2% in the control group ($p < 0.001$); – the implementation rate of the pharmaceutical recommendation 80%
Cortejoso L et al. [8] (prospective study)	1,859 consecutive patients admitted to an orthogeriatric ward or discharged from an orthogeriatric ward or discharged from the geriatric day unit	– to evaluate the clinical significance of the detected medication errors; – interventions regarding medication reconciliation written at admission to the ward or orally by a pharmacist	– most of the pharmacist's interventions due to clinically significant interactions (30.4% n=308); – orthogeriatric ward: A total of 2,389 administration explanations and 252 medication plans written, explained, and given to the patients at discharge; – geriatric day unit: 48 medication plans written, and 447 explanations for the administration of the drugs conducted

Study	Sample Size (Inclusion/exclusion criteria Included)	Objective/Methodology	Results
Denneboom W et al. [9] (randomised, controlled trial)	738 older (≥75 years) patients with polypharmacy (>5 drugs) Exclusion criteria: – terminally ill, – living in a home for older people	– to determine which procedure (case conferences or written feedback) leads to more medication changes (savings and costs were included); – pharmacists performed treatment reviews in both cases conference consulted and written feedback consulted intervention groups of older people with polypharmacy; reviews conducted via computerised screening tool and enhanced by pharmacists, then passed on to GPs	– pharmacists in the case-conference group identified significantly more recommendations themselves than the pharmacists in the written-feedback group (41.7% vs 34.2%, P = 0.003); – significantly more medication changes initiated in the case-conference group than in the written-feedback group (42 vs 22, P = 0.02); – this difference still present 6 months after treatment reviews (36 versus 19, P = 0.02) but 9 months after treatment reviews, the difference was no longer significant (33 vs 19, P = 0.07); – additional costs in the case-conference group covered by the greater savings in this group
Rose O et al. [10] (cluster-randomised controlled study)	142 patients Inclusion criteria: – age 65 years or older, – ≥ 3 chronic diseases out of 2 different organ systems with at least 1 cardiovascular disease, – use of 5 or more systemically available drugs, – given formal consent to participate in the study, – history of ≥ 1 visit to the GP during each of the past 3 quarters of the year Exclusion criteria: – insufficient ability to speak or read German, – participation in other studies, – severe, end-stage diseases (probable death within 12 months)	– to identify and prioritise eligible patients for a medication review and create criteria for patient selection; – measured acceptance of the prescribing physician's acceptance of the pharmacist's recommendation; – Intervention: after GPs provided all medical data concerning patients and home-care specialists conducted an interview and a classification, pharmacists performed a medication review and transferred it to GPs for potential therapeutic changes	– the chance of benefiting from a medication review rises by 1.06 per 1-point increase in the baseline MAI score; – per each discrepancy between the prescribed and the used drugs, the chance for a major benefit from the medication review increases 1.2 times; – the earlier patients entered the intervention period, the more beneficial it was for them; – a total of 366 (54.9%) of the 667 drug therapy recommendations were implemented by the physicians; – reasons for non-acceptance were the need for further information (18%), medical reasons (9%), budgetary reasons (5%) or special aspects in the patient's treatment history unknown to the pharmacist (68%)

Table 2: Summary of interventions associated with interprofessional collaboration between physicians and pharmacists from the PubMed database

Study	Cooperation between pharmacist and primary physician
De Bock L et al. [5]	The medication review was followed by a discussion between the pharmacist and the geriatrician; additional meetings between the geriatrician and pharmacist to discuss recommendations and acceptance
Köberlein-Neu J et al. [6]	After reviewing the patient's data, the pharmacist passed medication management recommendations to the primary care physician who provided feedback on the implementation of recommendations
Nachtigall A et al. [7]	All issues regarding a patient were discussed during a meeting with the physician, which was arranged by a pharmacist. Discussion preceded acceptance, modification or rejection of the recommendations. In only 20 cases, a face-to-face meeting was not feasible, however, a consultation was conducted by placing recommendations into patient's charts.
Cortejoso L et al. [8]	Drugs at admission and proposed alterations were registered electronically by the pharmacist so the primary physician was aware of possible problems with medications. In many cases, the pharmacist was requested by a physician or a nurse for information related to the pharmacotherapy.
Denneboom W et al. [9]	The pharmacist and GP discussed all recommendations during case conferences regarding individual patients, completing a standardised care plan together.
Rose O et al. [10]	GP provided a feedback form for the pharmacist's recommendation regarding the analysed patient. No face-to-face discussion between the pharmacist and GP was mentioned in the study protocol.

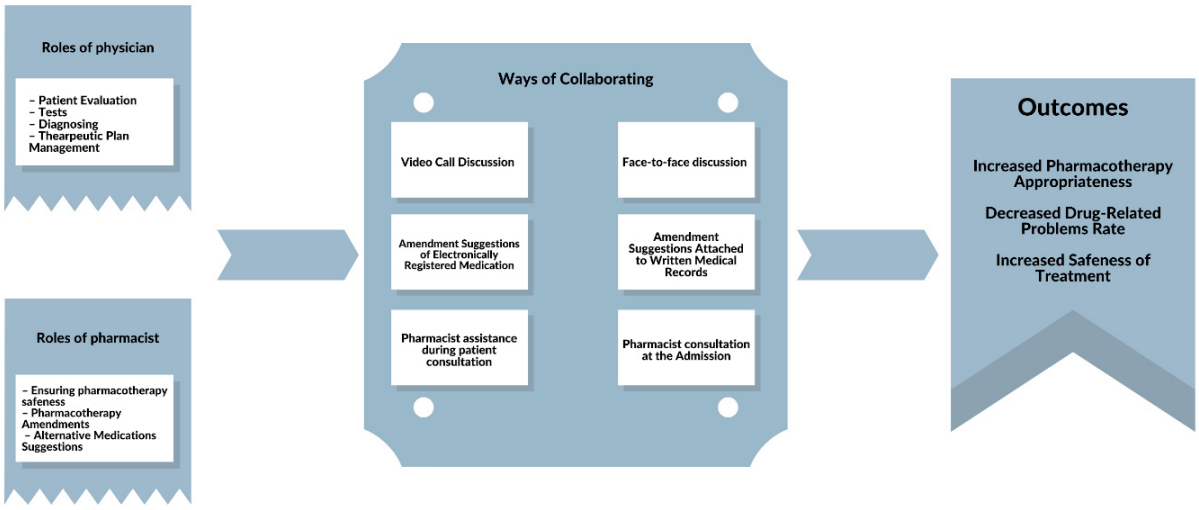

Figure 1. Methods of collaboration involving physicians and pharmacists regarding their roles and potentially positive outcomes in medication therapy in older patients. This graphic was composed by the authors

gain a benefit from the assistance of the pharmacist. This support may be especially prevalent in the potential of involving polypharmacy. Avoiding the possibility of drug-based interactions can only enhance the outcome and wellbeing of patients. Additionally, counselling can be provided with the use of technological resources, such as telemedical services, to restrict the spread of coronavirus among healthcare professionals and patients [15].

Conclusions

Collaboration between physicians and pharmacists has proven to positively impact the care and clinical outcomes of elderly patients. Indeed, interdisciplinary approaches are slowly becoming critical in the daily problem-solving of cases encountered in contemporary medicine.

The vast majority of medical specialisations, including geriatrics, requires consistent knowledge from a variety of disciplines such as pharmacokinetics, pharmacodynamics, interactions, indications and contraindications, dosing and adverse effects of drugs. Application of this knowledge in daily therapeutic routines is challenging but becomes crucial in consideration of polypharmacy in older people. The importance of considering an interdisciplinary education, especially within the field of medicine, should be the fundamental focus of any medical-based educational system.

The COVID-19 pandemic has overexerted healthcare systems throughout the world, with pharmacists and physicians often placed on the frontline against the pandemic. Pharmacists have proven to be a valuable asset in healthcare services with contributions to chronic disease management and drug-based interactions. Their specialised knowledge, when implemented in the treatment process of COVID-19, can not only enhance the wellbeing of patients but also alleviate the burden placed on the healthcare system.

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Conflict of interest statement
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References

1. Zermansky AG, Alldred DP, Petty DR, Raynor DK, Freemantle N, Eastaugh J, Bowie P. Clinical medication review by a pharmacist of elderly people living in care homes—randomised controlled trial. Age and Ageing. 2006 Aug 12;35(6):586-591. https://doi.org/10.1093/ageing/afl075
2. Makowsky MJ, Schindel TJ, Rosenthal M, Campbell K, Tsuyuki RT, Madill HM. Collaboration between pharmacists, physicians and nurse practitioners: A qualitative investigation of working relationships in the inpatient medical setting. Journal of Interprofessional Care. 2009 Jan;23(2):169-184. https://doi.org/10.1080/13561820802602552

3. Tonna AP, Stewart D, West B, McCaig D. Pharmacist prescribing in the UK – a literature review of current practice and research. Journal of Clinical Pharmacy and Therapeutics. 2007 Dec;32(6):545-556. https://doi.org/10.1111/j.1365-2710.2007.00867.x
4. Schellens JHM, Grouls R, Guchelaar HJ, Touw DJ, Rongen GA, de Boer A, Van Bortel LM. The Dutch model for clinical pharmacology: collaboration between physician- and pharmacist-clinical pharmacologists. British Journal of Clinical Pharmacology. 2008 Jul;66(1):146-147. https://doi.org/10.1111/j.1365-2125.2008.03156.x
5. De Bock L, Tommelein E, Baekelandt H, Maes W, Boussery K, Somers A. The Introduction of a Full Medication Review Process in a Local Hospital: Successes and Barriers of a Pilot Project in the Geriatric Ward. Pharmacy. 2018 Feb 28;6(1):21. https://doi.org/10.3390/pharmacy6010021
6. Köberlein-Neu J, Mennemann H, Hamacher S, Waltering I, Jaehde U, Schaffert C, Rose O. Interprofessional Medication Management in Patients With Multiple Morbidities. Deutsches Aerzteblatt Online. 2016 Nov 4;. https://doi.org/10.3238/arztebl.2016.0741
7. Nachtigall A, Heppner HJ, Thürmann PA. Influence of pharmacist intervention on drug safety of geriatric inpatients: a prospective, controlled trial. Therapeutic Advances in Drug Safety. 2019 Jan;10:204209861984336. https://doi.org/10.1177/2042098619843365
8. Cortejoso L, Dietz RA, Hofmann G, Gosch M, Sattler A. Impact of pharmacist interventions in older patients: a prospective study in a tertiary hospital in Germany. Clinical Interventions in Aging. 2016 Sep;Volume 11:1343-1350. https://doi.org/10.2147/cia.s109048
9. Denneboom W, Dautzenberg MG, Grol R, De Smet PA. Treatment reviews of older people on polypharmacy in primary care: cluster controlled trial comparing two approaches. Br J Gen Pract. 2007 Sep;57(542):723-31. PMID 17761060
10. Rose O, Mennemann H, John C, Lautenschläger M, Mertens-Keller D, Richling K, Waltering I, Hamacher S, Felsch M, Herich L, Czarnecki K, Schaffert C, Jaehde U, Köberlein-Neu J. Priority Setting and Influential Factors on Acceptance of Pharmaceutical Recommendations in Collaborative Medication Reviews in an Ambulatory Care Setting – Analysis of a Cluster Randomized Controlled Trial (WestGem-Study). Cignarella A. PLOS ONE. 2016 Jun 2;11(6):e0156304. https://doi.org/10.1371/journal.pone.0156304
11. Hedima EW, Adeyemi MS, Ikunaiye NY. Community Pharmacists: On the frontline of health service against COVID-19 in LMICs. Research in Social and Administrative Pharmacy. 2020 Apr;. https://doi.org/10.1016/j.sapharm.2020.04.013
12. Zheng S, Yang L, Zhou P, Li H, Liu F, Zhao R. Recommendations and guidance for providing pharmaceutical care services during COVID-19 pandemic: A China perspective. Research in Social and Administrative Pharmacy. 2020 Mar;. https://doi.org/10.1016/j.sapharm.2020.03.012
13. Aruru M, Truong H, Clark S. Pharmacy Emergency Preparedness and Response (PEPR): a proposed framework for expanding pharmacy professionals' roles and contributions to emergency preparedness and response during the COVID-19 pandemic and beyond. Research in Social and Administrative Pharmacy. 2020 Apr;. https://doi.org/10.1016/j.sapharm.2020.04.002
14. McCreary EK, Pogue JM. Coronavirus Disease 2019 Treatment: A Review of Early and Emerging Options. Open Forum Infectious Diseases. 2020 Mar 23;7(4). https://doi.org/10.1093/ofid/ofaa105
15. Moazzami B, Razavi-Khorasani N, Dooghaie Moghadam A, Farokhi E, Rezaei N. COVID-19 and telemedicine: Immediate action required for maintaining healthcare providers well-being. Journal of Clinical Virology. 2020 May;126:104345. https://doi.org/10.1016/j.jcv.2020.104345

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Chapter in the book

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