ORIGINAL ARTICLE

Prognostic impact of *NOTCH1, MYD88*, and *SF3B1* mutations in Polish patients with chronic lymphocytic leukemia

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KEY WORDS

chronic lymphocytic leukemia, *NOTCH1*, *SF3B1*, *MYD88*, stereotyped subsets

ABSTRACT

INTRODUCTION Currently available prognostic factors determining the course of chronic lymphocytic leukemia (CLL) are not fully efficient, especially for newly diagnosed patients. Investigation of molecular changes may help clarify the reasons for the heterogeneity of the disease. Apart from already confirmed *TP53* mutations, the novel candidates: *NOTCH1, SF3B1,* and *MYD88* might represent clinically relevant biomarkers.

OBJECTIVES The aim of this study was to evaluate the mutational status of *NOTCH1, MYD88*, and *SF3B1* and to compare the results with confirmed prognostic factors: ZAP-70, CD38, and immunoglobulin heavy-chain variable region (*IGHV)* mutation in CLL. The study assessed also prognostic significance in terms of the time to first treatment (TTFT) and subset analysis.

PATIENTS AND METHODS The study was conducted on samples of 370 newly diagnosed patients with CLL. The analysis was performed using high-resolution melting, Sanger sequencing, and polymerase chain reaction methods.

RESULTS Patients harboring the *NOTCH1* mutation were significantly more often found among patients with an unmutated *IGHV* gene status and high expression of CD38 and ZAP-70. The *MYD88* mutation was equally distributed in patients with mutated and unmutated *IGHV* status (5 vs 7 patients). For *MYD88* and *SF3B1,* there were no significant differences in the levels of CD38 and ZAP-70 expression. The tendency for lower median TTFT was revealed in patients with mutated *SF3B1* ($P = 0.08$). The analysis showed the presence of 14 different types of the subsets of *IGHV* in 50 of 345 patients (14.5%). The most frequent were subsets #1 and #2.

CONCLUSIONS The *NOTCH1* and *SF3B1* mutations accompany biological markers of unfavorable prognosis in patients with CLL. The mutations may contribute to the identification of patients with high-risk CLL.

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INTRODUCTION Chronic lymphocytic leukemia (CLL) is a highly heterogeneous disease due to the variety of genetic and epigenetic changes involved in its development. The clinical course of CLL varies from stable to rapidly progressive, with the survival of patients ranging from 1 year to 15 years or more.[1-4] Despite the considerable improvement in the understanding of the pathophysiology of CLL over the past decade, its pathogenesis has not been precisely defined.[1] Detection of certain biological markers might help determine the possible course of the disease. Moreover, recent findings showed that approximately one-third of patients may be grouped according to repetitive sequences in the immunoglobulin heavy-chain variable region (*IGHV*) which forms B-cell receptor (BCR). The presence of almost identical BCR immunoglobulin

TABLE 1 Clinical characteristics of patients

Number of patients		370
Sex	Female	150
	Male	220
Age, y, median (range)		65 (32–90)
Rai stage	0	124
	I	53
	II	68
	II	15
	IV	43
	Not available	67
ZAP-70 (cut-off 20%)	Positive (≥20%)	110
	Negative (<20%)	204
	Not available	56
CD38 (cut-off 30%)	Positive (≥30%)	93
	Negative (<30%)	229
	Not available	48
IGHV gene mutation status	Mutated	175
	Unmutated	192
	Not available	3

Abbreviations: *IGHV,* immunoglobulin heavy-chain variable region; ZAP-70, zeta-chain-associated protein kinase 70

in unrelated patients is defined as stereotyped subset. The subsets are characterized by strikingly similar biology of tumor cells, and patients assigned to the same stereotyped subset exhibit a similar disease course and outcome.[5] Nevertheless, currently available prognostic factors do not fully clarify the clinical heterogeneity and the molecular pathogenesis of CLL and are not always efficient in predicting the course of the disease, especially when it is diagnosed at an early stage.[6]

Recently, the next-generation sequencing technologies revealed previously unknown somatic mutations such as neurogenic locus notch homolog protein 1 (*NOTCH1*), myeloid differentiation primary response gene 88 (*MYD88*), and splicing factor 3B subunit 1 (*SF3B1*), which might represent the new biomarkers of prospective clinical relevance in CLL.[7-10] The described mutations have been observed in CLL cells with frequency accounting from 2% to 18%.[10-12] In the case of *NOTCH1* and *SF3B1,* mutations were detected more often among patients with progressive or high-risk CLL, while the role of *MYD88* has not yet been fully elucidated.[11,12] According to Rossi et al,[13] combining these new mutational data with the Döhner cytogenetic model results in better stratification of patients depending on the risk of progression and substantially increases the ability to predict survival. The 2016 revision of the World Health Organization classification suggested that these mutations have a potential clinical relevance and could be integrated into an updated cytogenetic risk profile.[14] However, a prognostic model created in 2016 by the Chronic Lymphocytic Leukemia – International Prognostic Index (CLL-IPI) Working Group included only the *TP53* status, implying that other recurrent genetic abnormalities do not show independent prognostic information.[15] By focusing on this purpose, researchers have taken the effort to verify the utility of novel mutations as prognostic factors. However, high heterogeneity of the disease requires further studies on various groups of patients.

In our study, we focused on mutations of unclear clinical significance, and thus assessed mutational status of *NOTCH1, MYD88,* and *SF3B1* in a representative group of patients. We analyzed the results in comparison with the currently available prognostic factors: zeta-chain-associated protein kinase 70 (ZAP-70), CD38, *IGHV* gene mutational status, and *IGHV* subsets. We also assessed prognostic significance in terms of the time to first treatment (TTFT). Our study showed the negative impact of the *NOTCH1* and *SF3B1* mutations.

PATIENTS AND METHODS

Characteristics of patients The study included 370 newly diagnosed and previously untreated patients with CLL, each of whom was tested for at least one of the evaluated genes. The cohort included 150 women and 220 men at a median age of 65 years (range, 32–90 years). Distribution of disease stages according to the Rai classification was as follows: stage 0, 124 patients; stage I, 53 patients; stage II, 68 patients; stage III, 15 patients; and stage IV, 43 patients. Clinically, the prognostic significance in terms of the TTFT was assessed for 202 patients with CLL. The TTFT was defined as the time from diagnosis to the start of treatment, in accordance with the criteria for treatment proposed by the International Workshop on Chronic Lymphocytic Leukemia.[16] The detailed clinical characteristics of patients are presented in **TABLE 1**.

Ethical approval was granted by local review committees and informed consent was collected according to the principles laid by the Declaration of Helsinki.

Peripheral blood mononuclear cells and DNA isolation Peripheral blood samples were collected from 370 newly diagnosed and previously untreated patients with CLL. Peripheral blood mononuclear cells were isolated using Ficoll density gradient centrifugation (Biochrom, Berlin, Germany). For DNA preparation, QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) was used according to the manufacturer's instructions. DNA was quantified by BioSpec-nano (Shimadzu, Kyoto, Japan).

Amplification refractory mutation system for *NOTCH1* and *MYD88* mutations *NOTCH1* c.7544_7545delCT (n = 316) in the PEST domain (exon 34) and *MYD88* L265P (n = 323) mutations were investigated by amplification refractory mutation system polymerase chain reaction (PCR). Primer sequences are described in Supplementary material online (*Table S1*). Reactions for *NOTCH1* were

performed in a 20-µl reaction volume containing the following: multiplex, primer for the wild-type gene sequence, primer for the mutated gene sequence, and reverse primer. Reactions for *MYD88* were performed in 2 samples, a 20-µl reaction volume each; the first sample contained multiplex, forward primer, and reverse primer for the wild-type gene; the second sample contained multiplex, forward primer, and reverse primer for the mutated gene. PCR products were separated by gel electrophoresis.

High-resolution melting analysis and Sanger sequencing for *SF3B1* mutations Screening for *SF3B1* (n = 364) mutations K700, E622/R625, and H662/K666 (exons 14 and 15) was performed using high-resolution melting (LightCycler 480, Roche, Basel, Switzerland). Primer sequences are described in Supplementary material online (*Table S1*). Amplified fragments were 87 base pair (bp), 119 bp, and 51 bp in length. The reaction mixture consisted of forward and reverse primers for the *SF3B1* gene and SsoFast EvaGreen® supermix (BIO-RAD, Hercules, California, United States), which is a ready-to-use reaction cocktail consisting of deoxynucleotide triphosphates, Sso7d-fusion polymerase, magnesium chloride, EvaGreen dye, and stabilizers. Melting curves were compared with negative control consisting of DNA with the wild-type *SF3B1* gene, and with positive control consisting of DNA with established mutation. Samples of DNA with melting curve differing from the positive control were considered suspicious, and the presence of mutations was confirmed by Sanger sequencing.

SF3B1 mutations (K700, E622/R625, and H662/K666) were confirmed by Sanger sequencing. *SF3B1* exons 14 and 15 were sequenced using the forward and reverse primers. Primer sequences are described in Supplementary material online (*Table S1*). Amplified fragments were 419 bp and 372 bp. PCR reactions were performed in a 20-µl reaction volume containing the following: 10 pmol of each primer forward and reverse, and 1 × Qiagen multiplex PCR Kit (Qiagen, Hilden, Germany). PCR products were used as templates for sequencing in both directions. Direct sequencing was performed using the Big Dyes Terminator v1.1 Cycle Sequencing kit (Applied Biosystems, Foster City, California, United States) and Applied Biosystems 3500 Genetic Analyzer (Applied Biosystems).

***IGHV* mutation status and subset analysis** The *IGHV* mutation status was determined by amplification with PCR. Six different sense primers specific for the framework region (FR) 1 consensus family (*IGHV*1-*IGHV*6) and 1 antisense primer complementary to the germline JH regions were used according to the BIOMED-2 Concerted Action protocols.[17] Primer sequences are described in Supplementary material online (*Table S1*). The seven *IGHV* families were amplified with 6 individual PCRs. PCR products were separated by electrophoresis on 2% agarose gels, cut out, and purified using a QIAquick Gel Extraction Kit (Qiagen). PCR products were used as templates for sequencing in both directions using an ABI PRISM BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems) on an automatic ABI 3500 Genetic Analyzer (Applied Biosystems), following the manufacturer's instructions. The percentage of hypermutation was calculated. Each clonal DNA *IGHV* sequence was aligned with the closest germline sequence using the international immunogenetics information system (http://www.imgt.org/). The sequences with a germline homology of 98% or higher were considered as unmutated, and those with a homology less than 98%—as mutated. The type of gene rearrangement was also determined.

A subset analysis was performed with the use of ARRest/AssignSubsets software (http://tools.bat.infspire.org/arrest/assignsubsets/).[18] The software enables the robust assignment of BCR IG sequences from patients with CLL to the 19 major subsets in CLL. The subset characteristics used to create this tool were based on the study of Agathangelidis et al.[19]

Flow cytometry analysis for ZAP-70 and CD38 The expression of ZAP-70 and CD38 was assessed with flow cytometry. Based on a CD-38 cut-off value of 30%, patients with CLL were categorized into a CD38-positive (≥30%) or a CD38-negative subgroup (<30%). For ZAP-70, the cut-off value was defined as 20% and patients were categorized into a ZAP-70-positive (≥20%) or a ZAP-70-negative subgroup (<20%). Methodology is described in more detail in Supplementary material online.

Statistical analysis Statistical analyses were performed using GraphPad Prism 5 (La Jolla, California, United States). The Mann–Whitney and Kruskal–Wallis tests were used to evaluate the differences between the subgroups. The correlations of variables were computed with the Spearman rank correlation coefficient. The Kaplan–Meier method was used to assess TTFT of patients with CLL. Survival curves were compared using the log-rank test. Statistical significance was defined as a P value of less than 0.05.

RESULTS

***NOTCH1* mutations** *NOTCH1* c.7544_7545delCT mutation occurred in 19 of 316 patients (6.0%) with CLL. Patients harboring the *NOTCH1* mutation were significantly more often characterized by the unmutated *IGHV* gene status (n = 18 of 19, 94.7%) than patients with the wild-type *NOTCH1* gene (P <0.0001). The mutation was significantly more often associated with high level of CD38 expression. It accounted for 10 of 18 CD38-positive cases (55.0%) among individuals with the *NOTCH1* mutation in comparison with 66 of 250 cases (26.4%) with the wild-type gene (P = 0.0132). The expression of ZAP-70 was also significantly higher in individuals with

TABLE 2 Analysis of association between *NOTCH1, MYD88,* and *SF3B1* mutations and prognostic factors in chronic lymphocytic leukemia

Parameter	n/N	%	n/N	%	*P* value
	NOTCH1 mutated		*NOTCH1* wild-type		
Unmutated *IGHV* status	18/19	94.7	146/293	49.8	<0.0001
CD38 positive	10/18	55.0	66/250	26.4	0.01
ZAP-70 positive	12/18	66.0	77/242	31.8	0.004
Subset #1	2/19	10.5	9/298	3.0	NS
Subset #2	0/19	0.0	10/298	3.4	NS
Male sex	11/19	57.9	176/294	59.9	NS
	MYD88 mutated		*MYD88* wild-type		
Unmutated *IGHV* status	7/12	58.3	162/308	52.6	NS
CD38 positive	1/12	8.3	78/265	29.4	NS
ZAP-70 positive	4/12	33.3	89/257	34.6	NS
Subset #1	0/12	0.0	9/265	3.4	NS
Subset #2	1/12	8.3	11/265	4.2	NS
Male sex	7/12	58.3	184/308	59.7	NS
	SF3B1 mutated		*SF3B1* wild-type		
Unmutated *IGHV* status	12/17	70.6	177/343	51.6	NS
CD38 positive	6/13	46.2	84/303	27.7	NS
ZAP-70 positive	6/12	50.0	101/296	34.1	NS
Subset #1	1/17	5.9	11/337	3.3	NS
Subset #2	2/17	11.8	8/337	2.4	NS
Male sex	13/17	76.5	203/346	58.7	NS

Abbreviations: *NOTCH1*, neurogenic locus notch homolog protein 1; NS, not significant; *MYD88*, myeloid differentiation primary response gene 88; *SF3B1*, splicing factor 3B subunit 1

mutated *NOTCH1* gene (n = 12 of 18, 66%) than in those with wild-type *NOTCH1* gene (n = 77 of 242, 31.8%) (P = 0.0041). The hemoglobin level in *NOTCH1*-mutated patients (median, 12.4 g/dl) was significantly lower than in wild-type *NOTCH1* patients (median, 13.5 g/dl; P = 0.0046). Analysis of the *IGHV* subsets in patients with the *NOTCH1* mutation revealed frequent presence of subset #1 in 2 of 19 patients (10.5%). Patients belonging to subsets #5, #6, #201, and #202 were also identified, each in single *NOTCH1*-mutated CLL case (5.2%). The complete analysis of the association between the *NOTCH1* mutation and prognostic markers in CLL is presented in TABLE 2 and Supplementary material online (*Figure S1*).

***MYD88* mutations** The *MYD88* mutation occurred in 12 of 323 patients (3.7%) with CLL. *MYD88* mutations were nearly equally distributed in patients with mutated and unmutated *IGHV* status (5 vs 7). In the population of *MYD88*-mutated patients, 1 patient belonged to subset #2 and another—to subset #4. There were no significant differences in the levels of CD38 and ZAP-70 expression. The complete analysis of correlations between the *MYD88* mutation and prognostic markers in CLL is presented in TABLE 2 and Supplementary material online (*Figure S1*).

***SF3B1* mutations** *SF3B1* mutations occurred in 17 of 364 patients (4.7%) with CLL. Furthermore, 2 patients showed the negative prognostic features of subset #2 and 1 patient—of subset #1. Patients belonging to subsets #3 and #6 were also identified. There were no significant differences in the levels of CD38 and ZAP-70 expression. The complete analysis of correlations between the *SF3B1* mutation and prognostic markers in CLL is presented in TABLE 2 and Supplementary material online (*Figure S1*).

Stereotyped subsets The analysis exposed the presence of 14 different types of subsets in 50 of 345 patients (14.5%). The most frequent were: subset #1 representing 12 patients with cLL (3.5%) and subset #2 representing 11 patients (3.2%). These 2 subsets accounted for 24% and 22% of stereotyped CLL, respectively. The percentage of subsets #1 and #2 in the groups of patients harboring particular mutations are presented in TABLE 2. Furthermore, the analysis revealed the presence of subsets #3 (n = 2), #4 (n = 3), #5 (n = 3), #6 (n = 5), #7H (n = 1), #8 (n = 2), #28A (n = 1), #59 (n = 1), #64B (n = 2), #99 (n = 1), #201 (n = 2), and #202 (n = 4).

Clinical implications The assessment of median TTFT revealed the significant differences between patients from various prognostic groups (FIGURE 1). Patients with unmutated *IGHV* gene status were characterized by significantly shorter TTFT than patients harboring the mutation

(median 4 vs 65 months, P <0.0001). The similar correlation occurs in patients with ZAP-70 positive in comparison with ZAP-70 negative (median 11 vs 26 months, P = 0.04) and CD38 positive versus CD38 negative (median 4 vs 29 months, P = 0.0003). There were no significant differences in patients with mutated and unmutated *NOTCH1* and *MYD88*, while in patients harboring *SF3B1* mutation the tendency to lower median TFTT was revealed (median 10 vs 14 months, P = 0.08). Interestingly, the significant difference in median TTFT was observed in groups of men and women, showing the better outcome in female patients (median 10 vs 28 months, P = 0.01).

DISCUSSION Understanding CLL genetics contributes to clarifying the molecular bases of clinical heterogeneity of the disease. Since 2000, stratification of CLL patients is practicable thanks to the cytogenetic model presented by Döhner et al.[20] It focuses on chromosomal abnormalities, which are detectable in more than 80% of patients with CLL. However, it occurs that molecular lesions provide additional information that also may have prognostic impact. The 2016 revision of the World Health Organization classification of lymphoid neoplasms indicated the potential clinical relevance of molecular alterations such as *TP53, NOTCH1, SF3B1, ATM*, and *BIRC3*.[14] It has been suggested that some of these could be integrated into an updated cytogenetic risk profile that also includes the well-known recurrent chromosomal abnormalities typically identified with fluorescence in situ hybridization studies.[21] In 2016, the International CLL-IPI Working Group created a prognostic model combining genetic, biochemical, and clinical parameters. It includes 5 independent factors: age, clinical stage, β_2-microglobulin concentration, *IGHV* mutation status, and *TP53* status. Other than *TP53* disruption, recurrent genetic abnormalities, such as *NOTCH1* and *SF3B1*, did not show independent prognostic information.[15]

The prognostic potential of the molecular lesions *NOTCH1, MYD88,* and *SF3B1* has been confirmed by several research groups, but still has not been used in clinical practice.[10-13,21] These mutations are also believed to be a part of the mechanisms explaining the heterogeneity of the disease and might point to new potential therapeutic targets in CLL.[10]

To our knowledge, we were the first to assess the occurrence of the analyzed mutations in a large representative cohort of Polish patients. The frequency of the *NOTCH1* mutation in newly diagnosed patients with CLL reached 6.0% and was comparable to data provided by other investigators.[10,22] Similarly, *SF3B1* mutations were detected in 4.7% of the patients at diagnosis, which is in agreement with the findings of Rossi et al,[23] who reported the frequency of 5%. In fact, it has been shown that the frequency of the mutation can increase with disease progression, even up to 20% during relapse.[8,24] The *NOTCH1* and *SF3B1* mutations are associated with particularly poor prognosis. The comparison with the currently available prognostic factors showed the accumulation of negative markers in *NOTCH1*-mutated individuals. A significant association with the unmutated *IGHV* status was revealed, confirming the findings of previous studies.[10,24-27] Interestingly, *NOTCH1* mutations were especially common in patients with gene rearrangement type *IGHV*1-69 (6 of 36 patients with *IGHV*1-69 vs 19 of 310 patients with *IGHV*; P <0.05) that confirms the association demonstrated in the Chinese study.[28] Moreover, the *NOTCH1* mutation was significantly more often associated with positive ZAP-70 and CD38 expression, which is associated with poor prognosis. It was reported that the *NOTCH1* mutation was more frequent among patients in advanced clinical stage, mostly treated or enrolled in clinical trials.[21] The mutation has also a strong positive association with trisomy of chromosome 12 and negative association with isolated del(13q). Moroever, it has been associated with decreased overall survival in at least 2 studies,[10,22] but was not an independent marker of survival.[29]

Despite the accumulation of negative prognostic factors in patients with *NOTCH1* and *SF3B1* mutations, we did not demonstrate a significant shortening of the TTFT compared with wild-type individuals. However, the analysis revealed a tendency to shorter TTFT in patients harboring the *SF3B1* mutation. We did not demonstrate an association between *SF3B1* mutations and other negative prognostic factors. This partly corroborates the findings of Oscier et al,[21] who showed no association between the *SF3B1* mutation and high expression of ZAP-70 or *IGHV* gene mutation status, but demonstrated a correlation between the occurrence of mutation and high level of CD38 expression. The mutation was also positively associated with advanced clinical stage at diagnosis, unmutated *IGHV* genes, and del(11q), as well as negatively associated with trisomy of chromosome 12 and isolated del(13q).[21]

The present study did not confirm positive prognostic implications for the *MYD88* mutation, since the distribution of negative prognostic markers was similar in both mutated and unmutated individuals and no statistically important differences in the TTFT between those groups were shown. Our data argue with previous reports that pointed to more frequent cooccurrence of the *MYD88* mutation and mutated *IGHV* gene status.[30,31] However, the impact of this mutation on prognosis in CLL remains unclear. According to a study of Martinez-Trillos et al,[32] *MYD88* mutations were associated with a longer TTFT and overall survival. These data are in contrast with previous studies reporting no differences in the TTFT or overall survival for *MYD88*-mutated vs wild-type patients.[13,21,33] Likewise, the most recent study by Baliakas et al[34] questioned the prognostic implications of the *MYD88* mutation, while no significant differences were detected either in

the occurrence of genetic aberrations or the clinical outcome. Although the study showed a trend for a shorter TTFT in patients harboring the mutation, this difference could be attributed to the advanced clinical stage among *MYD88*-mutated individuals. Therefore, the relevance of the *MYD88* mutation as a prognostic factor remains unexplained and larger collaborative studies are required.

CLL is also characterized by stereotyped BCR immunoglobulins that can be assigned to a particular subset on the basis of shared sequence motifs within the variable heavy complementarity determining region 3. Stereotyped subsets display almost identical biology of the leukemic clones; moreover, they might have similar clinical consequences.[5] In our study, we confirmed the presence of 14 subsets accounting for 14.5% of this CLL cohort, in contrast to previous reports that showed a more frequent occurrence of up to 30% of BCR subsets in CLL.[35,36] This discrepancy is possibly due to the limitation of the ARRest/AssignSubsets software (used in our study for subset classification), which distinguishes only 19 major subsets.[5,18] Subsets #1 and #2 are associated with highly aggressive clinical course of the disease with a short TTFT.[5] Recent studies demonstrated that 20% of subset #1 CLL cases were characterized by *NOTCH1* mutations.[37,38] In our study, only 2 of 19 subset #1 CLL patients had *NOTCH1* mutations. We also observed the *SF3B1* mutation in patients with subset #2 in 2 of the total 11 cases, which is partly in contrast to previous reports indicating an association of almost 50%.[37] We reported 3 cases of subset #4 in the CLL cohort, one of which occurred together with the *MYD88* mutation, and the other cases with the absence of *NOTCH1* or *SF3B1* recurrent mutations, which is in line with other studies.[37] Furthermore, subset #4 is associated with indolent clinical course.[5]

Apart from molecular and cytogenetic lesions, some other novel immunological markers might be of prognostic significance.[39] In the current study, we also validated the prognostic value of other clinical parameters in terms of the TTFT. Interestingly, we found a significantly longer TTFT in women than in men, confirming the effect of sex on prognosis in CLL.[40-42] So far, the trend for better outcome in women has not been clarified. Three factors possibly contributing to these results were identified: the association with good prognostic factors, pharmacokinetic differences between the sexes, and the effect of estrogens.[40]

Summarizing, *NOTCH1* and *SF3B1* mutations are associated with certain biological markers of unfavorable prognosis. Undeniably, these mutations may contribute to the identification of patients with high-risk CLL, and in combination with conventional negative prognostic factors of the CLL-IPI, they might provide additional information on disease prognosis.

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Contribution statement KG and PW conceived the idea for the study and contributed to the design of the research. JZ, JP, EZ, AK, and ES were involved in data collection. MP, MP, MP, JK, JZ, JZ, JP, EZ, and AK were responsible for laboratory work and data analysis. MP, MP, MP, JK, and KG prepared the manuscript with complete data interpretation. All authors contributed to this work and approved the manuscript for submission.

Supplementary material online Supplementary material online is available with the online version of the article at www.pamw.pl.

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