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Intracellular neutrophil myeloperoxidase level in pediatric patients: significant age and gender variability

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SUMMARY

Introduction: Myeloperoxidase (MPO) is an enzyme produced by neutrophil leukocytes and released upon activation, killing pathogens and causing tissue damage. As neutrophils in neonates are functionally immature, cellular MPO content in children may be age-related and different from adults. Hematological analyzer ADVIA 2120i (Siemens AG), while routinely assessing neutrophil count, measures their MPO content by means of myeloperoxidase index (MPXI). No pediatric reference range for MPXI has been published.
Methods: We performed a retrospective study of MPXI values in 51 303 consecutive routine blood tests of nonhematologic pediatric patients, looking for age- and gender-related variability. Nonparametric statistical analysis (Spearman rho and Mann–Whitney *U*-test) was used for evaluation.
Results: Neutrophil MPO content was highly significantly lower during the first month of life ($P << 0.001$) and rose to average pediatric values by day 28. Unexpectedly, the study revealed a highly significant gender difference ($P << 0.001$), MPXI being lower in boys from birth to adulthood.
Conclusions: We found previously unreported highly significant age- and gender-related variations of neutrophil MPO in children in hospital setting. There are possible clinical implications, particularly concerning neutrophil immunity of neonates and gender-related difference in vascular events in adults. The finding may be considered for refining automatic cell counting in infants.

INTRODUCTION

Myeloperoxidase (MPO) is a lysosomal enzyme found in azurophilic granules of neutrophils. It appears at the earliest differentiation stages and is secreted during cell activation and phagocytosis [1]. The most prominent MPO function is production of hypochlorous acid from hydrogen peroxide and chloride anion during the neutrophil's oxidative burst. Hypochlorous acid is highly cytotoxic and is used by

the neutrophils to kill bacteria and other pathogens [2,3].

Myeloperoxidase possesses potent proinflammatory properties and may contribute directly to tissue injury [3]. Extensive studies have reported an association between secreted (extracellular) MPO levels and the severity of coronary artery disease; it has been suggested that MPO plays a significant role in the development of the atherosclerotic lesion and in rendering plaques unstable [4,5]. A possible role of extracellular MPO in pathogenesis of demyelinating diseases [6], depression [7] and cancer [8] is currently under scrutiny.

Myeloperoxidase release causes decrease in intracellular MPO content, so immature granulocytes in blood as a rule contain more MPO than mature ones, and intracellular MPO level is lower in activated neutrophils. Thus, the quantity of cellular MPO changes dynamically along with neutrophil maturation and activity, although clinical relevance of these changes is not clear [1,2]. Other known factors that influence intracellular MPO are hematologic tumors and chemotherapy [9,10]. Significantly lower levels of MPO in neutrophils have been shown on animal model in neonatal period and during severe bacterial infection [11]; we have not found literature references on studies of neutrophil MPO content in human pediatric population.

As MPO expression is restricted to myeloid cells, it could be utilized to specifically define leukocyte populations, such as measurement of intracellular MPO in differential cell count by automated hematological analyzers [11–13].

AIM OF THE STUDY

Neutrophils in infants are functionally immature [14,15], and this transitory functional deficiency and the subsequent maturation process could be reflected by cellular MPO content [16]. The fact must be recognized to correctly interpret automated blood counts obtained by peroxidase-based hematological analyzers in children of different age. However, no age-related norms of cellular MPO content have been published so far.

A retrospective analytical study was undertaken to analyze cellular MPO measurements in pediatric patients to reveal possible age- and gender-related variations.

MATERIALS AND METHODS

Analytical data

51 303 consecutive routine blood counts for 28 019 children admitted to Children's Clinical University Hospital between March 2011 and April 2013 have been performed at the Hospital Clinical Laboratory and retrospectively analyzed. Patients with hematological disorders and tumors were not included in the study to avoid artifacts caused by abnormal hemopoesis and chemotherapy.

Patients were 0 days–17 years old at the time of blood test (median 4.5 years), 15 004 were boys (27 385 measurements) and 13 015 girls (23 918 measurements).

Mean peroxidase index (MPXI) values and patients' age and gender were obtained from the database of the Children's University Hospital Clinical Laboratory ('Dialab' laboratory information system created by SIA Diamedica, Latvia).

IBM SPSS v.21 software (IBM, Armonk, NY, USA) was used for statistical analysis; correlations (Spearman rho) and Mann–Whitney *U*-test for comparative statistics (with *z*-score and two-tailed *P*) were calculated.

MPXI method for MPO measurement

Routine EDTA-treated blood tests were performed by hematological analyzer ADVIA 2120i (Siemens AG, Munich, Germany) that had been calibrated on regular basis according to the manufacturer's guidelines. During the analysis, ADVIA 2120i determines peroxidase activity in each cell by directly measuring tungsten light absorbance as cytochemically stained leukocytes flow through the light beam. Neutrophils, monocytes, and eosinophils form peroxidase positive population, whereas lymphocytes and basophils are peroxidase negative. Neutrophil cloud is further defined and discriminated from monocytes and eosinophils by combination of peroxidase content and cell optical properties.

The integral result of MPO activity measurements in the cells within the defined neutrophil region is computed as the MPXI. MPXI is calculated as MPXI = [(Mean Neutrophil Region MPO−Expected Staining Index)/Expected Staining Index] × 100, where the Mean Neutrophil Region MPO is the result

of the absorbance measurement in the neutrophil region. The Expected Staining Index is the expected MPO measurement result for an ideal standard neutrophil population; it is a technical constant and is maintained by regular calibration [9–13].

A negative MPXI value means that the sample cells contain less peroxidase than the ideal normal population; a positive value means higher MPO content compared to the ideal normal population. The normal MPXI range for adults is 0 ± 10; no age-related norms have been published.

RESULTS

Mean peroxidase index value in the whole population (median −0.4; mean -1.16 ± 5.89) was slightly below the accepted adult normal range.

Mean peroxidase index in the whole cohort highly significantly correlated with age. Further analysis showed that the correlation was actually misleading and was due to a highly significantly decreased MPXI in neonates (median −3.1 *vs.* −0.2 in older children; $z = -30.79$, $P << 0.001$). If patients under 1 month were excluded, the age correlation was no longer present (Table 1).

The age distribution of MPXI was nonlineal (Figure 1): after the sharp increase after birth, there was a peak at 1–3 years followed by another milder drop at age 4–6 and then a gradual increase till adolescence.

Table 1. MPXI correlations with age and gender, (Spearman *rho*)

	Age*	Gender†
All patients		
rho	0.056	0.079
P	<<0.001	<<0.001
<1 m		
rho	0.149	0.087
P	<0.001	<0.001
>1 m		
rho	−0.005	0.080
P	0.272	<<0.001

MPXI, mean peroxidase index.
*Age in weeks for neonates, months for the whole population and older patients.
†Assigned 1 for boys and 2 for girls.

Unexpectedly, a highly significant correlation of cellular MPO content with gender was found (Table 1), MPXI being higher in girls (median 0.0 *vs.* −0.7; $z = -17.97$, $P << 0.001$). The difference was clearly seen at all ages except 9 and 14 years (Figure 2).

To rule out possible artifacts due to prolonged hospitalization, only MPXI from 28 019 single measurements at admission was analyzed for age and gender variations. The difference between neonates and older children calculated only for primary patients was highly significant, too (median MPXI −2.8 *vs.* 0.1, $z = -21.13$, $P << 0.001$). A highly significant MPXI difference between genders was found by the analysis of primary cases as well (median 0.3 in girls *vs.* −0.4 in boys, $z = -13.50$, $P << 0.001$).

DISCUSSION

The study of pediatric patients revealed a prominent variability of cellular neutrophil peroxidase (measured as MPXI by hematological analyzer ADVIA 2120i) when a large data block of over 50 000 consecutive measurements on a single platform was analyzed.

Myeloperoxidase content was highly significantly lower in neonates 0–28-day old in comparison with older children, thus confirming previous data obtained for animal development [11]; another decrease was found at age 4–6 years. The biological basis of those fluctuations is not clear; the finding may indicate a transitory age-specific MPO deficiency or an excessive discharge under activation or both. Further studies that include analysis of clinical aspects and functional studies of neutrophils may help to explain the age-related variability and its relation to neutrophil-mediated responses in children of different age.

Low MPXI in neonates may influence discrimination of cell populations by peroxidase-based hematological analyzers and should be taken into account by testing algorithms. MPO content in older children is similar to the established adult level; thus, the routine testing approach is applicable to the majority of pediatric population.

The highly significant relation of MPXI and gender was an unexpected finding, as there is no data on such difference in adults. It could be only partly explained by older age of female patients (median age 49 months *vs.* 44 months in boys, difference nonsignificant), for the distinction was clearly seen in separate age groups

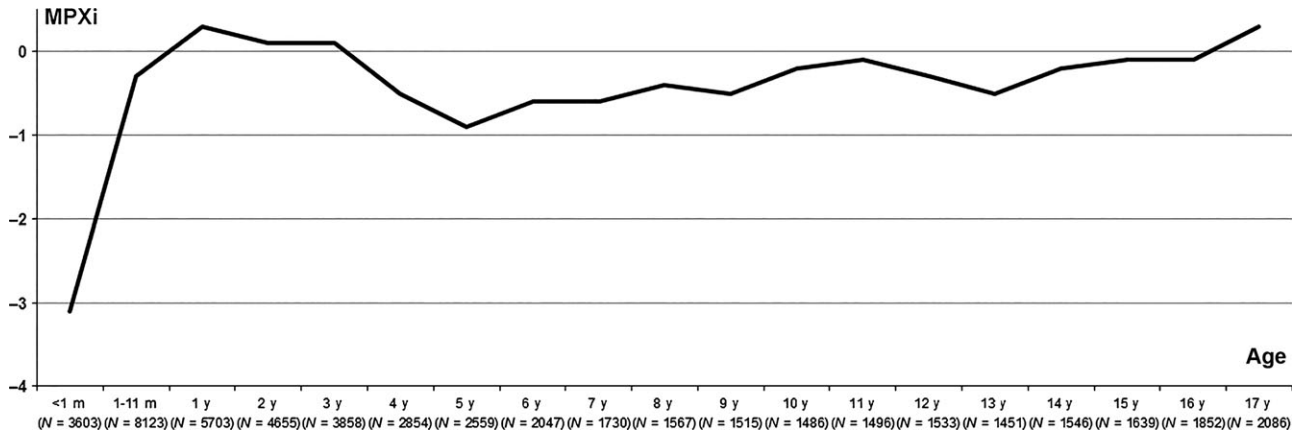

Figure 1. Median MPXI age-related variation.

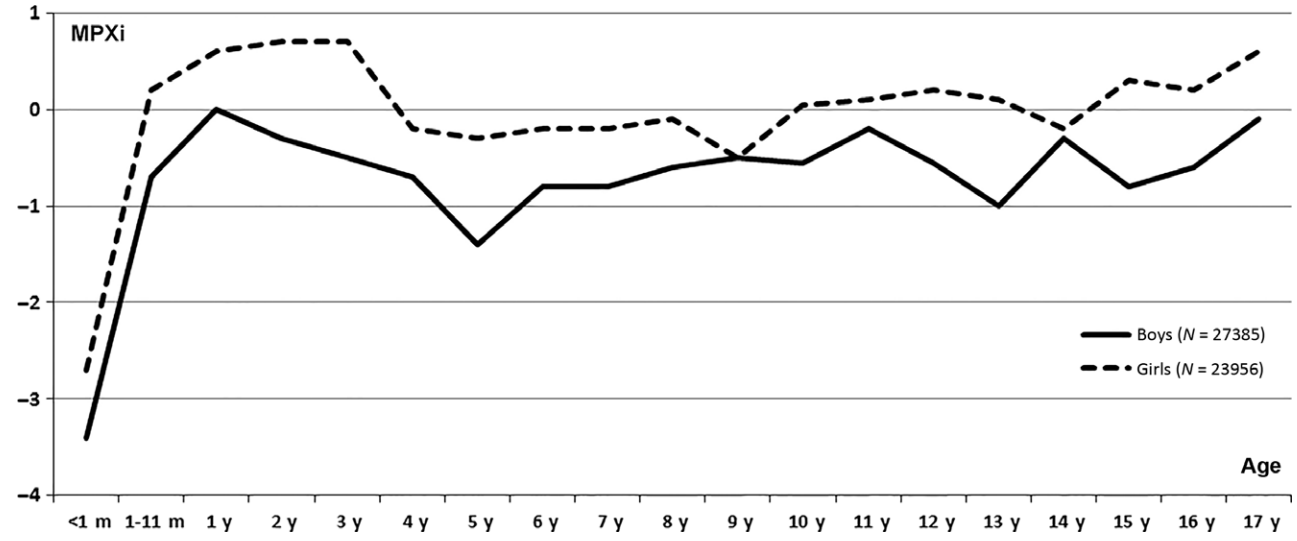

Figure 2. Median MPXI, in relation to age and gender.

(Figure 2). That gender-related difference should with high probability have a biological reason due to a very high degree of statistical significance, still, there seems to be no clear explanation for the phenomenon. Lower intracellular MPO content suggests more active release and thus higher level of extracellular MPO in males; if extrapolated, it might be a reason for gender-related differences in development of vascular damage at older age.

Treatment-related artifacts were excluded as a cause for the age and gender variations in MPXI by analyzing only blood samples at admission to the hospital, with similar results.

The study was carried out on cohort basis, no individual cases have been analyzed, and no correlations with clinical parameters were calculated. An additional study should elucidate a possible diagnostic or clinical value of variations in cellular MPO content; very high and very low MPXI values could be of particular interest. Further study of the phenomenon of low cellular MPO in neonatal patients seems to be indicated.

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