The Molecular Pathophysiology, Differential Diagnosis, and Treatment of MPO Deficiency

Rongqin Ren1, Yuri Fedoriw2 and Monte S. Willis2,3*

1Department of Pathology, East Carolina University, Greenville, NC, USA
2Department of Pathology & Laboratory Medicine, University of North Carolina, Chapel Hill, NC, USA
3McAllister Heart Institute, University of North Carolina, Chapel Hill, NC, USA

Abstract

Myeloperoxidase (MPO) deficiency is a common immune system variant affecting ~1:2000 people in the United States. MPO is partially responsible for the antimicrobial properties of both neutrophils and monocytes as a critical component in creating hypochlorous acid in phagosomes. While >95% of people with MPO are asymptomatic, a small percentage of people with MPO deficiency present with severe, often recurrent, microbial infections necessitating a clear understanding of its diagnosis and underlying pathophysiology. Primary “hereditary” MPO deficiency can be due to any number of recently recognized MPO mutations. Secondary or “acquired” causes, on the other hand, are also common due to a variety of exposures and concomitant diseases. The differentiation of primary and secondary causes can generally be made by assessing the clinical history and defining patterns of MPO expression. Generally, simple laboratory tests and/or clinical presentation and history can differentiate the underlying cause. No specific therapy is indicated in patients with MPO deficiency beyond microbe-specific therapy for their infections, along with strict control of blood glucose in diabetic patients.

Keywords: Myeloperoxidase; Pathophysiology; Recurrent infections; Phagocytes; Neutrophils; Laboratory testing; Molecular testing

Introduction

Myeloperoxidase (MPO) is an enzyme found in the azurophilic granules of neutrophils and in monocyte lysosomes, and is one of many enzymes involved in defense against common infections, such as fungi and bacteria. Myeloperoxidase was originally discovered in neutrophils in 1868 [1]; it was used in the differential diagnosis of myeloid and lymphoid leukemias beginning in 1916 [2] and first isolated in 1941 [3]. Beyond its use diagnostically, it was not until 1954, 1963, and 1966 that the first cases of human MPO deficiency were reported that could be localized to neutrophils and monocytes [4,5]. Initially this disease was believed to be quite rare; it wasn’t until large scale analysis of white cells using hemocytometers dependent upon MPO staining, was it realized that MPO deficiency is quite common. Complete MPO deficiency is found in 1 in 4000 people; partial MPO defects are found in 1 in 2000 individuals in the United States [6-9]. Despite this high prevalence, greater than 95% of people with this disorder are asymptomatic throughout their lifetime. Until recently, MPO deficiency has been classified as a primary immunodeficiency. However, in 2005, the International Union of Immunological Societies Primary Immunodeficiency Diseases Classification Committee omitted MPO deficiency because of its “marginal clinical relevance” [10]. However, in a small percentage of patients (<5%) with MPO deficiency, serious infections have been reported from Candida species, which can present as mucocutaneous, meningeal, bone, and even disseminated infections. Other studies have reported severe Staphylococcus aureus infections after injuries or surgical procedures and inflammatory diseases. Patients with diabetes mellitus or cancer have been linked to increased susceptibility to Candida infections in the context of MPO deficiency. So while MPO deficiency is rarely a problem, it potentially can be serious. In this review, we outline the detection of MPO deficiency in the laboratory, present an overview of the anti-microbial systems in phagocytic cells, and discuss the biosynthesis and process of MPO in the context of hereditary mutations. We then discuss the clinical manifestations and treatment of MPO deficiency, focusing on the differential diagnosis of similarly presenting diseases.

Detection of MPO Deficiency in the Laboratory

Until the 1970’s, only 15 cases of MPO deficiency had been reported. The use of automated flow cytometry and other modern laboratory techniques have allowed the screening of large study populations to determine a more accurate prevalence of MPO deficiency [11]. Patients with MPO deficiency are most commonly detected incidentally through routine screening tests in the clinical lab and present without any symptoms. The use of MPO staining was initially utilized in the determination of the cell lineages of immature cells in acute leukemias more than 30 years ago. Since MPO is found exclusively in the neutrophil lineage, the presence of MPO allows the differentiation of acute myeloid leukemia (AML) of granulocytic lineage from acute lymphoblastic leukemia and other AML’s of monocytic, erythroid, and megakaryocytic lineages. This staining characteristic has been adapted to many of the currently used automated hemocytometers, which use MPO staining to determine the WBC differential [12]. These automated systems stain white blood cells with 4-chloro-1-naphthol to label MPO, which are then analyzed by flow cytometry for size, complexity, and the presence of peroxidase activity [13]. Many hematology analyzers using this technique identify MPO deficiency by the identification of increased large unstained cells (LUCs), which are large cells with a low intensity of MPO staining. These large unstained cells can represent blasts, variant and atypical lymphocytes, or more commonly as patients...
with MPO deficiency, where their large neutrophils have decreased MPO activity. The presence of LUCs allows clinicians to differentiate the possibility of a hematological malignancy, activated lymphocytes, or the presence of MPO deficient patients in the larger clinical context.

Overview of Anti-microbial Systems in Phagocytic Cells

MPO is found in both neutrophils and monocytes. The original description of MPO was made by Agner [3], who identified this protein from the pus of a dog. His initially called it verdoperoxidase, since it gave both the pus and neutrophils their green color [3]. Neutrophil lysosomes predominantly contain MPO, in addition to other anti-microbial proteins. As such, MPO accounts for 2-3% of the total cellular protein in neutrophils (2-4 g per million cells) [14].

Neutrophils

Polymorphonuclear neutrophils (PMNs) are an important component of the innate immune system essential for optimal anti-microbial defense [15]. Mature PMNs contain two types of granules based on cytochemical staining: 1) peroxidase negative; and 2) peroxidase positive (azurophilic) granules [16]. The peroxidase-negative granules are composed of the formyl peptide receptor, fusogenic proteins (secretory carrier membrane protein and vesicle-associated membrane protein-2), β2 integrins, the NADPH oxidase component flavocytochrome b558, and urokinase-type plasminogen-activating receptor [17-22]. Peroxidase-positive (azurophilic) granules contain MPO, elastase and cathepsins (proteases), and other direct mediators of anti-microbial activity including azurocidin, defensins, component flavocytochrome b558, and urokinase-type plasminogen-activating receptor [17-22]. Peroxidase-positive (azurophilic) granules are taken up by granulocytes and placed into phagosomes, granules fuse and release their contents into the phagosome. NADPH produces mediators of anti-microbial activity including azurocidin, defensins, and bactericidal-permeability-increasing protein [16]. When pathogens are taken up by granulocytes and placed into phagosomes, granules fuse and release their contents into the phagosome. NADPH produces reactive oxygen species which are converted to H2O2 by superoxide dismutase, which kill ingested microbes in an oxygen-dependent manner (summarized in Figure 1A). MPO, a unique member of the peroxidase family, is capable of converting H2O2 to hypochlorous acid by oxidizing Cl⁻ to Cl⁺ (hypochlorous acid). The MPO-Hydrogen peroxide-CI system is thought to be a prominent response in the terminating the respiratory burst in neutrophils as individuals with MPO deficiency have prolonged respiratory bursts. The importance of the MPO reaction forming hypochlorous acid in pathogen killing is also highlighted in neutrophils from patients with MPO deficiency. MPO deficient neutrophils have a pathogen killing time that is twice as long compared to neutrophils from control patients in vitro [23]. MPO also functions in the production of tyrosyl radical production, chlorination, generation of tyrosine peroxide, mediates myeloid cell adhesion through a β2 integrin mechanism [24-27], and is involved in the oxidation of lipoproteins found in the serum, as discussed in more detail below.

Monocytes

Like neutrophils, monocytes contain MPO-positive granules in lysosomes. MPO-granules form during the promonocytic stage in the bone marrow, and are easily detectable in circulating monocytes, although there are generally only 1/3 the amount found in neutrophils [28]. As monocytes differentiate into macrophages in tissue, their MPO expression has been reported to be lost, which might be regained under certain conditions [29]. Macrophages in liver, microglia, and granule-containing neurons have also been reported to express MPO [30,31].

MPO Biosynthesis and Processing

Our understanding of the biosynthesis and processing of MPO has been characterized in some detail and offers a glimpse into the diversity of the underlying mechanisms, which lead to MPO deficiency [32-35]. Myeloperoxidase is encoded by a single gene (MPO) found at the 17q22-23 chromosome locus, which consists of 12 exons and 11 introns [36-38]. When translated, the MPO gene forms a peptide precursor, which consists of an amino-terminal signal peptide, a propeptide following, which is trialed by a small, then a large subunit [39]. The synthesized MPO protein is next glycosylated to form an enzymatically inactive pre-pro-MPO. The pre-pro-MPO is then folded, modified with oligosaccharides, and sorted into granule storage (Figure 1B). Pre-pro-MPO is glycosylated with oligosaccharides once on the pro-peptide region and four times on the heavy subunit [33]. Pre-pro-MPO binds to the chaperone calnexin and calreticulin to facilitate its folding in the endoplasmic reticulum; calreticulin facilitates the insertion of a heme group [40]. When heme is added, an enzymatically active precursor is formed, called the pro-MPO (Figure 1B) [41]. Pro-MPO spends a long time in the ER before export, a process independent of the chaperones calreticulin and calnexin, which generally are involved in ER quality control [42]. This may have to do with the time necessary to integrate heme into the protein. The pro-peptide acts to sort MPO into the granules (Figure 1B) [35]. Pro-MPO undergoes additional conversations to eventually become mature MPO; a process involving the formation of a disulfide bridge between 2 MPO proteins, forming the active mature dimer of MPO (Figure 1B) [35]. The mechanisms of the process of pro-MPO are not fully understood.

Hereditary “Primary” MPO Deficiency

In the research setting, multiple missense mutations in the MPO gene have been identified in patients with MPO deficiency (summarized in Figure 2). However, the identification of mutations is not part of the diagnosis of MPO deficiency. These studies have helped in elucidating how MPO is processed and may further inform the underlying mechanisms of MPO deficiency. These mutations also demonstrate the diversity of disease phenotypes, outlined below.

R569W

The substitution of tryptophan for arginine at codon 569 of the MPO gene has been one mutation described in patients with MPO deficiency. Compound heterozygotes, or the presence of 2 single mutations in R569W, have been most commonly identified in MPO deficiency patients the United States [43]. One person with homozygous (i.e. two) R569W mutations has been described. This missense mutation prevents the maturation of the pre-pro-MPO, resulting in enzymatically inactive protein that is not delivered to the azurophilic granules [43].

Y173C

The substitution of tyrosine for cysteine at codon 173 leads to misfolding of the protein, which is due to the additional intramolecular sulfide bonds that are formed. The Y173C protein is converted to pro-MPO, but misfolded at this step and sequestered by the chaperone calnexin and retained in the ER [44]. This prevents it from entering the secretory pathway, targeting it to the azurophilic granules. Protein quality controls systems, including the ubiquitin proteasome system, then degrade the Y173C protein at an accelerated rate [45].
Figure 1: Overview of oxygen-dependent anti-microbial systems in neutrophils/monocytes and the processing of myeloperoxidase. **A.** Myeloperoxidase (MPO) acts upon hydrogen peroxide to generate hypochlorous acid (HOCl) used to kill bacteria and fungi in the phagosome. The NADPH oxidase complex is pivotal in generating superoxide anion (O$_2^-$) from oxygen (O$_2$), using NADPH generated by glutathione (GSH) pathway. Superoxide dismutase (SOD) then converts superoxide into hydrogen peroxide (H$_2$O$_2$), which is the substrate for MPO to create hypochlorous acid (HOCl). Both H$_2$O$_2$ and HOCl are directly involved in the oxygen-dependent anti-microbial systems (see text for details). **B.** Myeloperoxidase (MPO) undergoes a multistep process before reaching the phagosomes or being secreted. The pre-pro-MPO protein is translated as a single precursor. During the process of being folded, oligosaccharide modifications are added to the protein. The pre-pro-MPO has one mannose oligosaccharide on the pro-peptide, and 4 are placed on the $\alpha$ (large) subunit [33]. This pre-pro-MPO is then folded with the help of chaperones, which facilitate the insertion of a heme group. It is then cleaved to form the pro-MPO. The pro-MPO peptide acts to sort MPO for further processing, where it undergoes the formation of a disulfide bridge allowing the active mature dimer to be made (see text for details).

**Abbreviations:** G6P: Glucose-6-Phosphate; GSH: glutathione; HOCl: Hypochlorous acid; MPO: Myeloperoxidase; NADPH: Nicotinamide Adenine Dinucleotide Phosphate.

Panel A adapted from van der Veen [85]. Panel B adapted from: Olsson et al. [35].
In Japanese populations, both R499C and G501S mutations lead to complete MPO deficiency, and may be due to their localization near H502, which is an important region for heme association [48,49]. These mutations may affect the structural stability of the MPO protein as their proximity to three heme sites appears to be important in maintaining structural integrity [50].

Other MPO mutations

Since hereditary MPO deficiency is the most common biochemical defect of neutrophils, recent larger population-based studies have begun to elucidate how common each of these mutations are. Approximately 40,000 Italian individuals were studied; 7 partial and 8 total MPO deficient patients were identified [51,52]. They found 3 previously identified mutations (T752C, C1705T, 1566_1579del14), 6 novel mutations, four missense mutations (C995T, A1112G, T1715G, R499C, G501S, R569W, S572W, W643A) have been identified in a single patient with complete MPO deficiency. As the scope of specific mutations that cause MPO become clearer, the use of directed molecular tests to identify specific mutations may become available. Currently, these tests will take more time and cost more than MPO activity tests.

Secondary “Acquired” MPO Deficiency

Secondary or “acquired” MPO deficiency can be differentiated from primary MPO deficiency by the variability of MPO activity in neutrophils and medical history (Table 1). Multiple medical conditions have been associated with secondary MPO deficiency, in which a partial deficiency is seen, generally in a subset of neutrophils [53]. These conditions include lead intoxication [54], iron deficiency, severe infection [55], thrombotic diseases [56], renal transplantation, diabetes mellitus [6,57,58], drugs, disseminated cancers, as well as hematological disorders and neoplasms of the developing granulocyte lineage [53]. These include myeloid leukemias (acute and chronic AML, CML), polycythemia vera [59], Hodgkin disease, refractory anemia, aplastic anemia, primary myelofibrosis, and myelodysplastic syndromes [53,60]. While the underlying mechanisms of many of these causes are not completely delineated, a few of them have been more clearly defined. For example, lead intoxication inhibits heme synthesis, which is a necessary component in the maturation of MPO [53]. Additionally, severe infections can cause PMNs to be activated, leading to the consumption of MPO, which then appears as an MPO deficiency [53]. Deficiency in MPO in the secondary “acquired” forms is transient and improves with resolution of the primary illness. Most patients with an acquired form of MPO deficiency only have a fraction of their cells affected; that is, they have cells present with full MPO activity. However, in patients with myeloproliferative disorders, the deficiency may affect several granulocyte lines [61,62]. In the setting of iron deficiency, iron replacement of is necessary for MPO biosynthesis to continue [63].

Clinical Manifestations, Differential Diagnosis, and Treatment of MPO Deficiency

The most common infections in MPO deficient patients are from different Candida species, which can present as mucocutaneous, meningeal, bone, and disseminated fungal infection [9,64-68]. Some patients with complete MPO deficiency can also develop severe Staphylococcus aureus infections after injuries or surgical procedures, and inflammatory diseases such as spondylarthritides [11]. Patients with diabetes mellitus or cancer are more susceptible to Candida infections in the context of MPO deficiency [6,69-72]. Many of these reports

Table 1: Differentiation of primary and secondary MPO deficiency. Adapted from Lanza et al. [53].
associating infection risk in MPO deficiency were published in the 1970’s and 1980’s, although they continue to be published. More recent data suggests that there may be less of an association of MPO deficiency with increased infections than previously thought.

In patients with these symptoms, a fairly broad differential diagnosis should be considered in the context of the patient’s presentation. The differential diagnosis should include Chronic granulomatous disease (CGD), Glycogen-Storage Disease Type 1 (GSD1), Hyperimmunoglobulinemia E Syndrome, Kostmann Disease, Leukocyte Adhesion Deficiency, and Schwachman-Diamond Syndrome. All of these disorders have the recurrent life-threatening bacterial and fungal infections in common, however, they differ in their broader spectrum of disease (Table 2). For example, chronic granulomatous disease can only be differentiated by laboratory tests as it affects the same killing pathways as MPO deficiency. This contrasts to Schwachman-Diamond syndrome in which pancreatic insufficiency and skeletal abnormalities often overshadow the neutrophil deficiency that can be associated with recurrent infections. The often simple clinical and laboratory distinction from MPO is summarized in Table 2. The definitive diagnosis of MPO deficiency is made by histochemical staining of neutrophils for myeloperoxidase [73]. No specific therapy is indicated in patients with MPO deficiency beyond microbe-specific therapy for their infections, along with strict control of blood glucose in diabetic patients.

### How Can Most MPO Patients be Asymptomatic?

Greater than 95% of patients lacking MPO are asymptomatic, despite studies of human MPO deficient neutrophils indicating they have abnormalities in killing Candida albicans and hyphal forms of Aspergillus fumigatus [74,75]. Despite the lack of MPO, MPO deficient neutrophils retain much of their ability to kill a variety of microbes. Initially, S. aureus, Serratia spp., and E. coli killing is impaired, but reaches normal levels after time [76]. This suggests alternative but overlapping mechanisms may compensate in this context.

Much of what is known about the differential killing ability in MPO deficiency comes from MPO-knockout mice. Like neutrophil depleted mice, MPO knockout mice have a high susceptibility to infection with C. albicans [77-79]. There is considerable more susceptibility to pulmonary infections after intranasal challenge with C. tropicalis, Trichosporon asahii, and Pseudomonas aeruginosa, and to a lesser degree Cryptococcus neoformans, Klebsiella pneumoniae, and Aspergillus fumigates [78,80,81]. However, there is no increased

<table>
<thead>
<tr>
<th>Summary</th>
<th>Distinction from MPO Deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic Granulomatous Disease (CGD)</td>
<td>An inherited disorder of phagocytic cells. Inability of phagocytes to produce superoxide anions (O2·). Recurrent life-threatening bacterial and fungal infections. Caused by defect(s) in the NADPH oxidase complex.</td>
</tr>
<tr>
<td>Glycogen-Storage Disease Type 1 (GSD1)</td>
<td>Patients have large livers with excessive glycogen. Patients present with hypoglycemia, lactic acidosis, hypertiglyceridemia, and hyperuricemia. A common manifestation of this disease is neutropenia, putting the patient at risk of increased infection.</td>
</tr>
<tr>
<td>Hyperimmunoglobulinemia E (Job) Syndrome</td>
<td>Characterized by recurrent skin abscesses, pneumonia, eczematous dermatitis, and elevated IgE levels (&gt;10 times normal values). Both autosomal dominant and recessive forms have been described. Caused by mutations in the STAT3 and DBCM8 genes. Caused by abnormal neutrophil chemolaxis due to decreased IFNγ by T lymphocytes.</td>
</tr>
<tr>
<td>Kostmann Disease</td>
<td>Rare disease characterized by decreased neutrophil counts (ANC&lt;200/µl). Severe persistent neutrophilia results in susceptibility to bacterial infections. The autosomal recessive form is caused by mutations in the MAX1 genes; the autosomal dominant form is caused by mutations in the neutrophil elastase gene (ELS2).</td>
</tr>
<tr>
<td>Leukocyte Adhesion Deficiency (LAD)</td>
<td>Rare primary immunodeficiency characterized by marked leukocytosis and localized bacterial infections. Caused by a failure to express CD18, which composes the CD11a/CD18 (LFA-1) expressed on lymphocytes and antigen presenting cells.</td>
</tr>
<tr>
<td>Schwachman-Diamond Syndrome (SDS)</td>
<td>Rare autosomal recessive disorder characterized by pancreatic insufficiency, bone marrow dysfunction, and skeletal abnormalities. Symptoms of malnutrition secondary to pancreatic insufficiency predominate. Caused by mutations in the SBDS gene believed to be involved in RNA metabolism or ribosome assembly. SDS patients have a low neutrophil count that leaves patients at risk of developing severe recurrent infections, along with anemia and thrombocytopenia. Bone marrow typically is hypocellular with maturation arrest of all myeloid lineages.</td>
</tr>
</tbody>
</table>

| Table 2: Differential diagnosis in patients presenting with recurrent infections. Adapted from: Rosenzweig and Holland [73] and Petersen and Sheikh [86]. |

susceptibility in MPO-deficient mice to C. glabrata, S. aureus, and S. pneumonia [81]. The finding that there is no susceptibility to S. aureus is consistent with observations make with MPO deficient human neutrophils, where early, but not late killing of S. aureus is impaired, but long term killing is not affected [76].

MPO-deficient mice are variably susceptible to E. coli depending on the exposure. Challenging MPO-deficient mice with sepsis induced by cecal ligation and puncture results in enhanced infection (103). However, challenging MPO-deficient mice with E. coli intraperitoneally resulted in a reduction of lung infections [82]. This study identified that MPO-deficient mice had an increased expression of vessel inducible nitric oxide synthase (iNOS) in their lungs and neutrophils, resulting in a 2-6 fold increased in NO production compared to mice with MPO [82]. The enhanced NO is able to react with O$_2^-$ to form ONOO$^-$, which can kill microbes (Figure 1A) and may compensate for the decreased HOCl-mediated killing of bacteria in MPO deficient mice [82]. The number of neutrophils in mice is only 10-20% of that found in humans [83,84], so mice are normally less reliant on neutrophil killing and may not reflect what happens in humans. However, mice have been useful in delineating the non-MPO derived killing mechanisms that may be clinically relevant in understanding our defenses against different types of microbes [85].

Summary
Both complete and partial MPO deficiency is common, being present in 1:4000 and 1:2000 people in the United States, respectively. Despite this high prevalence, most patients are asymptomatic throughout their lifetime. It is this minimal clinical significance which led to the International Union of Immunological Society Primary Immunodeficiency Diseases Classification Committee to recently remove MPO deficiency from the classification as a primary immunodeficiency. However, a small percentage of people present with severe, often recurrent, microbial infections necessitating a understanding of its diagnosis and underlying pathophysiology. MPO is present in both neutrophils and monocytes and is a critical component in creating hypochlorous acid in phagosomes to secure the killing of microbes (summarized in Figure 1A). MPO can be defective due to mutations in MPO (Figure 2), which can result in improper processing (Figure 1B). Secondary causes are also common due to a variety of exposures and concomitant diseases, including lead intoxication, iron deficiency, severe infection, thrombotic diseases, renal transplantation, diabetes mellitus, drugs, disseminated cancers, as well as hematological disorders. The differentiation of primary and secondary causes can generally be made by assessing the clinical history and identifying different patterns of MPO expression (summarized in Table 1). Patients presenting with recurrent infections should be considered to have other, less common diseases, in addition to MPO (outlined in Table 2). Generally simple laboratory tests and/or clinical presentation and history can differentiate the underlying cause (Table 2). Currently, molecular diagnosis of MPO mutations is performed on a research basis only and not used clinically. No specific therapy is indicated in patients with MPO deficiency beyond microbe-specific therapy for their infections, along with strict control of blood glucose in diabetic patients.

References
and tyrosine peroxide as a result of tyrosyl radical scavenging by glutathione. Arch Biochem Biophys 323: 429-437.


73. Rosenzweig SD, Holland SM (2011) Myeloperoxidase deficiency and other enzymatic WBC defects causing immunodeficiency. Waltham, MA.


