Myeloperoxidase in immune mediated vascular disorders

CHAPTER 10

Summary, conclusions and general discussion

Summary and conclusions

Myeloperoxidase (MPO)

Myeloperoxidase is a heme containing peroxidase present in neutrophils and monocytes. It is able to catalyze hypochlorous acid (bleach) from hydrogen peroxide and chloride ions and is therewith an important enzyme in the host defense against bacteria, viruses and fungi[1]. MPO can, however, also damage host tissue by its production of reactive oxidants and can be involved in the oxidative modification of low-density lipoproteins (LDL)[2,3], the consumption of nitric oxide (NO)[4,5], and the activation of proteolytic enzymes[6-8]. MPO and MPO-derived oxidants therefore also play a significant (patho)physiological role in other non-infectious diseases including immune mediated vascular disorders, such as the primary small vessel vasculitides and atherosclerosis.

Vasculitis

The primary small vessel vasculitides comprise a group of diseases (table 1), separate from the medium and large sized vessel vasculitides. They are characterized by inflammation of the vessel wall of the smallest arteries, veins, and/or capillaries resulting in for instance glomerulonephritis and alveolitis, and also (epi)scleritis, mononeuritis multiplex, purpura and petechiae[9]. In most cases specific auto-antibodies like anti-neutrophil cytoplasmic antibodies (ANCA), anti-glomerular basement membrane (anti-GBM) antibodies, cryoglobulins, or IgA are found. ANCA are specifically associated with Wegener's granulomatosis, microscopic polyangiitis, Churg-Strauss syndrome and idiopathic pauci-immune necrotizing glomerulonephritis. In these diseases, ANCA are mostly directed against either proteinase 3 (PR3) or MPO[10,11]. Anti-GBM antibodies are associated with anti-GBM crescentic nephritis, anti-GBM alveolitis, or pulmonary hemorrhage combined with crescentic glomerulonephritis (*i.e.* Goodpasture disease)[12]. Interestingly, about 30% of patients with anti-GBM mediated disease are positive for (MPO-)ANCA as well.

Atherosclerosis

Atherosclerosis is the underlying cause for heart attacks, strokes, and peripheral vascular disease and is the major cause of death in western society. It is a vascular disease involving primarily the large and medium sized vessels (>3mm external diameter)[13]. Central in one of the major theories trying to explain the pathogenesis of atherosclerosis, the so-called response to injury theory by Ross, is the oxidative modification of LDL and endothelial cell dysfunction[14].

The first part of this thesis (chapter 2 to 5) has focused on the role of MPO as an antigenic target. In **chapter 2** we reviewed the role of ANCA and anti-GBM antibodies in the diagnosis and follow-up of patients with vasculitic disease. ANCA and anti-GBM antibodies have proven to be important specific and sensitive diagnostic markers in vasculitic disease.

In contrast to anti-GBM mediated disease, ANCA-associated vasculitides are chronic diseases with a high relapse rate and since morbidity is dictated by the frequency and severity of these relapses, much health benefit can be achieved when a relapse is detected as early as possible.

Table 1. The primary small vessel* vasculitides[9].

Disease	Characteristics	(Auto-)antibody associated
-Henoch-Schönlein purpura	Vasculitis predominantly of the skin, joints, gastrointestinal tract, and kidneys. Mostly occurring in childhood with an average age of onset of 4 years. Characterized by palpabel purpura, colicky abdominal pain, vomiting, bloody diarrhea, melaena, macroscopic haematuria, and arthralgias.	IgA
-Mixed essential cryoglobulinemic vasculitis	Vasculitis secondary to mixed essential cryoglobulinaemia. Can be a primary disorder, or associated with haematological malignancies, infections, and/or autoimmune rheumatic diseases such as Sjögren's syndrome. In the last decade, a strong association of mixed essential cryoglobulinaemia with the presence of hepatitis C virus infections is recognized. Characterized by purpura, arthalgias, and nephritis.	Cryoglobulins
-Wegener's granulomatosis (WG)*	Granulomatous inflammation involving the respiratory tract and necrotizing vasculitis affecting small to medium-sized vessels in other organs. Characterized by nose bleeds, nephritis, lung infiltrates, and hemoptysis.	ANCA
-Churg-Strauss syndrome (CSS)*	Eosinophil-rich and granulomatous inflammation involving the respiratory tract and necrotizing vasculitis affecting small to medium-sized vessels. Characterized by nephritis, lung infiltrates and neuropathy. Associated with asthma, and eosinophilia.	ANCA
-Microscopic polyangiitis (MPA)*	Necrotizing vasculitis affecting small vessels with absence of granulomatous inflammation of the respiratory tract. Pulmonary capillaritis often occurs. Characterized by nephritis, purpura, and hemoptysis	ANCA
-Idiopathic pauci-immune necrotizingcrescentic glomerulonephritis (iNCGN)	Crescentic glomerulonephritis, with few or no immune deposits. Absence of small vessel vasculitis in other organs. Characterized by nephritis, and malaise.	ANCA
-Goodpasture disease-Isolated anti-GBM nephritis-Isolated anti-GBM alveolitis	Crescentic glomerulonephritis and/or alveolitis. Characterized by hemoptysis, nephritis, and malaise.	Anti-GBM antibodies (and in 30% of cases ANCA)

*small vessels include capillaries, venules, or arterioles

The literature showed that increases in ANCA titers and persistently high ANCA levels indicate a high risk of relapse and warrant clinical evaluation of the patient for signs of relapse.

In **chapter 3** we evaluated the use of a rapid ANCA-GBM dot-blot screening assay in terms of sensitivity, specificity, and inter-observer effect for the differential diagnosis of rapidly progressive glomerulonephritis (RPGN). RPGN may be caused by ANCA-associated crescentic glomerulonephritis (CGN), immune-complex induced glomerulonephritis, or anti-GBM CGN and is characterized by rapid and progressive loss of renal function and the presence of CGN. Prompt diagnosis is thus crucial in preventing extensive organ damage or even death. We concluded that the ANCA-GBM dot-blot is a useful screening tool in situations where conventional ANCA testing is not readily available with excellent performance for PR3-ANCA detection, but less optimal sensitivity for MPO-ANCA and specificity for anti-GBM detection.

In **chapter 4** we measured the affinity of anti-GBM antibodies of patients with anti-GBM mediated vasculitis for their purified antigenic target within the GBM, *i.e.* the α3 chain of the non-collageneous part of type IV collagen [α3IV(NC1)]. We postulated that the binding kinetics of the anti-GBM antibodies contributed to the clinically observed fulminant nature and treatment resistance of this disease. By comparison with other antibody interactions, these anti-GBM antibodies demonstrated high affinity (4.5 nmol/L), with relatively high on (binding) rates and slow off (dissociation) rates. The results suggested that anti-GBM antibodies bind rapidly and remain tightly bound to the GBM *in vivo*. This property likely contributes to both the fulminant nature of the disease and its resistance to therapy, because persistent glomerular antibody deposition has the potential to produce continuous inflammation, despite removal of circulating antibodies by plasmapheresis and adequate immunosuppression.

A substantial proportion (±30%) of patients with anti-GBM antibody mediated CGN are positive for MPO-ANCA as well (*i.e.* double positive). In **Chapter 5** we reviewed the occurrence of double positivity for our Limburg renal biopsy registry and evaluated the influence of the respective auto-antibodies on renal biopsy features. We found double positivity in 43% of our patients with anti-GBM mediated CGN. In their renal biopsies, double-positive CGN patients showed features of both MPO-ANCA associated CGN and anti-GBM nephritis. Renal and patient survival analysis revealed that, contrary to the literature, double positives had a worse prognosis than anti-GBM antibody positive patients, whereas renal survival in double positives was similar to anti-GBM CGN patients and worse compared to MPO-ANCA positive patients. We postulated that MPO-ANCA positivity predisposes to the development of anti-GBM CGN.

Chapter 6 to 8 focus on functional intra-neutrophil MPO levels in health and disease. MPO levels in neutrophils are influenced by the amount of MPO protein synthesized in the cell as well as by the amount that is released. In **Chapter 6** we studied the influence of two MPO promoter polymorphisms, MPO463 and MPO129, on the intra-neutrophil MPO activity and protein content in a large group of healthy individuals. We found that the 129GA polymorphism affects the MPO activity in neutrophils, the A allele being associated with lower MPO activity. For the 463GA polymorphism only a gender dependent difference in MPO activity in older age groups could be observed.

In **Chapter 7** we evaluated the influence of these two polymorphisms on large artery stiffness as measured by pulse wave velocity in a group of hypertensive patients. We hypothesized that an 'increased MPO' genotype results in an increased NO consumption with subsequent increase in pulse wave velocity. For the MPO129 polymorphism we found no differences, possibly due to the low number of heterozygous patients available. However, for the MPO463GG versus GA/AA genotype significant differences were observed in women, but not in men.

Patients with small vessel vasculitis often have evidence of neutrophil activation with release of MPO and recently, plasma levels of circulating MPO have been associated with disease activity[15]. In **Chapter 8** we investigated the intra-neutrophil MPO activity in a group of patients with small vessel vasculitis in remission compared to healthy controls. We also monitored the intra-neutrophil MPO activity during relapse of these patients. Although hampered due to the limited availability of patients, we observed a trend towards a lower MPO activity in women in remission, but not in men. Sequential measurements revealed that the standard deviation in patients in remission was greater than that of controls. In most patients with a relapse MPO activity decreased.

Finally, in **Chapter 9** we reviewed the current knowledge on the role of MPO in the pathogenesis of systemic vasculitis. MPO-ANCA can activate (primed) neutrophils directly causing extensive reactive oxygen species formation and degranulation of neutrophil constituents, including MPO, resulting in an aggravated immune response towards the vessel wall. MPO-ANCA can prevent the clearing and inactivation of MPO by ceruloplasmin as well, resulting in increased myeloperoxidase activity. Active myeloperoxidase may contribute to endothelial dysfunction and add to the chronic renal lesions observed in patients with MPO-ANCA. MPO levels are further influenced by hormonal and genetic factors including the MPO463 and MPO129 promoter polymorphisms. The 463 polymorphism has been associated with an increased risk of development of MPO-ANCA associated disease.

General discussion

Both vasculitis and atherosclerosis are characterized by an immune mediated vessel directed inflammatory process with an influx of MPO containing myeloid cells, and in both diseases the effects of MPO and its oxidative products are relevant to pathogenesis.

MPO as antigenic target

For long, no definitive proof was available that auto-immunity to MPO causes the pathogenic effects observed in MPO-ANCA associated vasculitis. The presented evidence was merely circumstantial. MPO-ANCA were found to be closely related to the presence of vasculitic disease. Relapses were often, but not always, associated with a rise in ANCA titer (reviewed in chapter 2). Also, *in vitro* evidence showed that MPO-ANCA were capable of activating primed neutrophils and promote neutrophil mediated endothelial cell injury (for review[16]). Moreover, in rats injection of anti-MPO antibodies to rats with sub-clinical nephritis caused marked disease aggravation, but MPO-ANCA could not by themselves cause disease[17]. However, during the

completion of this thesis, for the first time direct *in vivo* pathogenic proof has emerged. With the availability of the MPO knockout mouse it was possible to bypass tolerance and develop a full-blown species specific anti-MPO response in previously healthy animals. Transfer of anti-MPO antibodies to wild-type animals resulted in vasculitic disease similar to that observed in humans[18].

This animal model creates the opportunity to further decipher the pathogenic events important in the development of disease. Recently, our laboratory has shown that lipopolysaccharide (LPS), a bacterial component capable of priming neutrophils and promoting endothelial cell adhesion molecule expression[19], aggravates disease[20]. It will be interesting to test new therapeutic agents aimed at reducing disease severity. Agents that can prevent neutrophil activation and degranulation would be interesting candidates. In this respect, the administration of for instance relaxin, a peptide hormone, could result in a new therapy. Relaxin has been shown to inhibit both the LPS-induced adhesion of neutrophils to coronary endothelial cells as well as the activation of neutrophils by a NO mediated mechanism[21,22].

MPO levels

MPO levels in neutrophils are influenced by the amount of MPO protein synthesized in the cell as well as by the amount that is released. Increased MPO levels can promote MPO-mediated pathogenic processes, it has for instance been demonstrated that coronary atherosclerosis is associated with an increased neutrophil MPO content[23]. Also, mice genetically modified to over-express human MPO in their monocytes displayed increased atherosclerosis compared to wild type mice[24].

As we demonstrated (chapter 6), two MPO promoter polymorphisms influence intra-neutrophil MPO activity. Both polymorphisms have been linked to an increased risk for atherosclerosis and/or vasculitic disease[25-30]. However, for the MPO463 polymorphism data are often difficult to interpret since effects of this polymorphism on neutrophil MPO levels depend on age and gender. Moreover, both polymorphisms explain only a limited proportion of the observed variation in intra-neutrophil MPO activity, when measured in healthy individuals. Since MPO activity in these individuals was stable over time, other (un)identified (genetic) factors determine intra-neutrophil MPO contents as well, like gender (chapter 6). We found that intra-neutrophil MPO levels are higher in women than in men, whereas Hoy *et al* found the contrary in plasma MPO levels[31]. This apparent contradiction can be explained by the fact that in vitro studies have shown that estrogens can inhibit MPO degranulation and as such may result in higher intracellular MPO activity and lower MPO plasma levels in women than men[32]. Moreover, a clinical study has shown that the administration of estrogens to males prior to an elective coronary artery bypass operation lowers MPO release shortly after surgery[33].

In addition to genetic factors, intra-neutrophil MPO levels are also influenced by neutrophil age. Young neutrophils or band forms, recruited from the bone marrow contain more MPO than senescent neutrophils[34]. This suggests that neutrophils release or degrade MPO while ageing.

Finally, intra-neutrophil MPO content reflects neutrophil activation as well. Buffon *et al* showed that neutrophils, while passing through an atherosclerotic vasculature, were partially depleted of their MPO content[35].

Thus, intra-cellular MPO levels have been useful for evaluating the effect of two promoter polymorphisms, but in disease intra-neutrophil MPO levels not only reflect genetic differences, but also neutrophil recruitment and activation. When using MPO as a biomarker for neutrophil activation, it may be interesting to measure MPO serum levels since MPO serum levels originate mostly from neutrophil MPO. Recent data support the use of serum MPO as a biomarker of neutrophil activation. It has been shown that levels of circulating MPO correlate with disease activity in patients with Wegener's granulomatosis[15]. Also in atherosclerosis MPO levels have been associated with disease activity[23,36]. Baldus *et al* showed that, in patients with acute coronary syndromes, MPO serum levels could help predict the chance of death and myocardial infarction during 6 months of follow-up. Importantly, MPO serum level was a risk factor independent from previously established risk factors such as C-reactive protein, vascular endothelial growth factor, soluble CD40 ligand and troponin T. Patients with an elevated MPO serum level (above 350 ug/l) had twice the risk at death or non-fatal myocardial infarction at 6 months after inclusion[36]. These results were confirmed by Brennan *et al*[37].

However, MPO is more than an indicator of a neutrophil mediated inflammatory response, MPO itself is an active participant of pathogenic processes in various diseases. Clinically, it will be interesting to evaluate the effect of reducing MPO activity on disease severity and outcome.

Reducing MPO activity

Theoretically, there are several ways to reduce MPO activity. One could reduce the amount of bio-available MPO by reducing the amount of neutrophils and monocytes. This can be accomplished by removal of circulating neutrophils or by decreasing the production of neutrophils. Case reports have shown that the removal of circulating neutrophils might be of benefit in patients with MPO-ANCA associated vasculitis[38]. The inhibition of neutrophil production (with for instance cyclophosphamide) is part of the standard treatment of MPO-ANCA associated vasculitis. The main disadvantages of these therapies are the numerous side-effects, which reduces long term applicability and the clinical usage in atherosclerotic disease.

Another way to reduce MPO activity is by preventing neutrophil degranulation. Since degranulation is preceded by neutrophil priming and activation, agents preventing these events also prevent MPO release. Examples include general anti-inflammatory agents (*e.g.* steroids), TNFα inhibitors (etanercept, infliximab), estrogens, and other cytokine mediating agents. Steroids are routinely and successfully used to treat MPO-ANCA associated vasculitis, but not atherosclerotic disease. The use of TNFαinhibitors is experimental, but they may be additionally effective in ANCA associated vasculitis disease[39]. Strikingly, they may also help to reduce endothelial dysfunction in patients with ANCA associated vasculitis, thus linking anti-vasculitic therapy to anti-atherosclerotic therapy[40]. Interestingly, a small clinical study has shown that the administration of estrogens to males prior to an elective coronary artery

bypass operation lowers MPO release shortly after surgery[33], indicating that estrogens inhibit MPO release as well.

A totally different approach to reduce MPO activity is to decrease MPO expression at a gene level. Oligonucleotide based therapies (*e.g.* small interfering RNAs and anti-sense oligonucleotides) aimed at specifically inhibiting MPO production are exciting potential therapeutic options[41]. Also, recently Reynolds *et al* have demonstrated that peroxisome proliferator-activated receptor-αgamma (PPAR-α) agonists (*e.g.* thiazolinediones clinically used as antidiabetic agents) strongly regulate MPO gene expression likely due to interference with the MPO promoter at the site of the MPO463 promoter polymorphism[42]. In this study *in vitro* gene expression was either strongly upregulated or downregulated depending on GM- or M-CSF co-incubation. *In vivo* studies should be performed to clarify the effect on MPO expression in disease.

Finally, MPO activity can be reduced by inhibitors of MPO. The most potent inhibitor of MPO is 4-aminobenzoic acid hydrazide[43]. It binds to the enzyme and irreversibly inactivates MPO. However, relative high concentrations of hydrogen peroxide are necessary to initiate this process, this likely limits the usefulness *in vivo*. A more specific and potent inhibitor is desirable to investigate the involvement of myeloperoxidase in inflammatory processes[1]. Methimazole, already clinically used to inhibit thyroid peroxidase in thyroid disease, also inhibits MPO. Commonly described 'side effects' include agranulocytosis. These side effects appear to be dose-related[44]. Unfortunately, no information is available about the *in vivo* concentrations necessary to inhibit MPO functions.

In conclusion, MPO is an important marker of and active participant in vasculitis and atherosclerotic disease. Measuring MPO in both diseases will be useful in establishing disease risk. MPO levels can identify and determine disease severity and disease activity during patient follow-up. In the future, it will be interesting to evaluate the use of MPO inhibiting agents as part of the treatment of both atherosclerosis and ANCA associated vasculitis.

Treatment of vasculitic disease consists of rapid intervention with heavy drugs, suppressing the acute inflammatory reactions. Treatment of atherosclerotic disease, however, is mostly directed towards chronic management of disease. Since many pathologic events overlap, therapeutic interventions used in atherosclerosis are potentially useful in vasculitic disease, and vice versa.

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Nederlandse samenvatting

Myeloperoxidase

Myeloperoxidase (MPO) is een enzym aanwezig in twee soorten witte bloedcellen, de neutrofielen en monocyten. MPO kan bleekwater genereren uit waterstofperoxide en chloride ionen. Hiermee is het een belangrijk enzym in de verdediging van het menselijk lichaam tegen bacteriën, virussen en schimmels. De door MPO gemaakte stoffen zijn echter zeer reactief en beschadigen soms ook het lichaam zelf. Zo kan MPO bijvoorbeeld het LDL cholesterol oxideren, stikstof monoxide verbruiken en proteolytische enzymen activeren.

Deze eigenschappen maakt dat MPO niet alleen belangrijk is bij de afweer tegen infecties, maar ook een belangrijke pathofysiologische rol speelt in immuungemedieerde vaataandoeningen, zoals de vasculitiden en arteriosclerose.

Vasculitiden

Vasculitis is een ontstekingsproces van bloedvaten resulterend in beschadiging en uiteindelijk destructie en afsluiting van deze vaten. Een vasculitis kan secundair aan een andere ziekte zijn (bijvoorbeeld reumatoïde artritis), maar komt ook primair voor. De verschillende vormen van primaire vasculitis worden meestal onderverdeeld in de grote, middelgrote en kleine vaten vasculitiden. De kleine vaten vasculitiden zijn een groep van ziektes gekenmerkt door ontsteking van de kleinste arteriën, venen en/of capillairen. Dit kan resulteren in bijvoorbeeld ontsteking van de nieren, longen, zenuwen, ogen, huid of andere organen. In de meeste gevallen van kleine vaten vasculitis worden in het bloed van de patiënt specifieke antistoffen zoals anti-neutrofiel cytoplasmatische antistoffen (ANCA), anti-glomerulaire basaal membraan antistoffen (anti-GBM), koude antistoffen, of IgA antistoffen gevonden.

ANCA zijn geassocieerd met de ziekte van Wegener, microscopische polyangiitis, het syndroom van Churg-Strauss en idiopathische pauci-immuun necrotizerende glomerulonefritis. In deze ziekten blijken ANCA meestal gericht te zijn tegen proteïnase 3 of MPO.

Anti-GBM antistoffen zijn geassocieerd met anti-GBM crescentische nefritis, anti-GBM alveolitis, of longbloedingen met crescentische glomerulonefritis (oftewel, de ziekte van Goodpasture). Interessant is dat er in ongeveer 30% van de patiënten met een anti-GBM gemedieerde ziekte ook antistoffen tegen MPO gevonden worden.

Arteriosclerose

Arteriosclerose is de onderliggende oorzaak voor hartaanvallen, herseninfarcten en perifere vaatziekten (bijvoorbeeld etalagebenen). Arteriosclerose is doodsoorzaak nummer 1 in de westerse samenleving. Het is een vataandoening van met name de middelgrote en grote vaten (>3mm in diameter). De op dit moment belangrijkste theorie die het ontstaan van arteriosclerose probeert te verklaren is de 'response to injury' theorie van Ross. Centraal in deze theorie is de continue beschadiging van de vaatwand door bijvoorbeeld roken, suikerziekte of hoge bloeddruk, het disfunctioneren van de vaatwandbekleding ('endothelial cell dysfunction') en de oxidatieve veranderingen van cholesterol partikels. MPO kan in deze processen een belangrijke rol spelen.

Het eerste gedeelte van dit proefschrift (hoofdstuk 2 tot 5) richt zich op de rol van MPO als antigeen voor ANCA. In hoofdstuk 2 beschouwen we de rol van ANCA en anti-GBM antistoffen in de diagnose en 'follow-up' van patiënten met vasculitis. ANCA en anti-GBM antistoffen zijn specifieke en sensitieve hulpmiddelen in diagnostiek naar vasculitiden. De literatuur laat zien dat een stijging in ANCA titer en persisterend verhoogde ANCA titers een vergroot risico op een recidief met zich meebrengt.

In hoofdstuk 3 hebben we de ANCA-GBM dot-blot test geëvalueerd wat betreft sensitiviteit, specificiteit en inter-waarnemer effect in de differentiaal diagnose van snel progressieve glomerulonefritis. Deze snel progressieve glomerulonefritis kan worden veroorzaakt door een ANCA-, immuuncomplex-, of anti-GBM gemedieerde ziekte. Onze conclusie was dat de ANCA-GBM dot-blot een bruikbare screeningsmethode is voor de detectie van deze antilichamen. Dit met name in situaties waar conventionele ANCA en anti-GBM testen niet beschikbaar zijn.

In hoofdstuk 4 hebben we de bindingssterkte (affiniteit) tussen anti-GBM antilichamen en de specifieke bindingsplaats voor deze antilichamen op de GBM bepaald. De binding was van een zeer hoge affiniteit, met snelle associatie en langzame dissociatie ratio's. Deze resultaten suggereren dat anti-GBM antilichamen snel en stevig binden aan de GBM *in vivo*. Deze eigenschappen dragen bij aan het ernstige en moeilijk te behandelen beloop van deze ziekte.

Een belangrijk deel (±30%) van patiënten met anti-GBM antistof gemedieerde glomerulonefritis hebben ook MPO antistoffen in het bloed, dat wil zeggen zijn dubbel positief. In hoofdstuk 5 hebben we gekeken naar het voorkomen van deze dubbel positiviteit in het regionale nierbiopten archief van Zuid-Limburg. Ook hebben we gekeken naar het effect van de verschillende auto-antistoffen op de nierbiopt karakteristieken. Wij vonden dat in Zuid-Limburg 43% van de patiënten met anti-GBM antistoffen ook positief waren voor anti-MPO antistoffen. Dubbel positieve patiënten hadden een slechtere overleving vergeleken met enkel positieve patiënten. De 'overleving' van de nier was in beide groepen echter gelijk. We postuleerden dat anti-MPO antistoffen predisponeren voor het ontwikkelen van anti-GBM gemedieerde glomerulonefritis.

Hoofdstuk 6 tot en met 8 gaan over de intra-neutrofiel MPO activiteit in zowel gezonde personen als in patiënten. De MPO hoeveelheid per neutrofiel wordt bepaald door de productie, afbraak en uitscheiding van MPO.

In hoofdstuk 6 bestudeerden we het effect van twee MPO promotor polymorfismen, MPO463 en MPO129, op de intra-neutrofiel MPO activiteit in een grote groep gezonde proefpersonen. We vonden dat in proefpersonen met het MPO129GA genotype de MPO activiteit significant lager was vergeleken met de MPO129GG genotypes. Voor het MPO463 polymorfisme vonden we leeftijd en geslachtsafhankelijke effecten. Dit maakt het noodzakelijk om in vergelijkende studies geslacht en leeftijd als variabelen mee te nemen.

In hoofdstuk 7 hebben we gekeken naar het effect van deze twee polymorfismes op de stijfheid van de grotere arteriën, gemeten middels de 'pulse wave velocity' in een groep van hypertensieve patiënten. Onze hypothese was dat een hoog MPO-gehalte genotype leidt tot een verhoogde stikstofmonoxide consumptie, resulterend in een stijver vaatbed. Voor het MPO129 polymorfisme vonden we geen verschillen, mogelijk als gevolg van het lage aantal beschikbare heterozygote patiënten. Voor het MPO464 polymorfisme daarentegen vonden we significante verschillen in vrouwen, maar niet in mannen.

In patiënten met een kleine vaten vasculitis wordt vaak bewijs gevonden van neutrofiel activatie met daarmee vrijkomen van MPO. In hoofdstuk 8 beschrijven we de intra-neutrofiel MPO activiteit in een groep van patiënten met een kleine vaten vasculitis, zowel tijdens actieve ziekte als gedurende remissie. Ondanks de relatief kleine groep van patiënten, vonden we een trend voor een lagere MPO activiteit in vrouwen met een vasculitis in remissie vergeleken met leeftijd en geslacht gematchte controles. Dit gold niet voor mannen. Opeenvolgende metingen lieten zien dat in vasculitis patiënten de activiteit vaak wisselde, dit in tegenstelling tot gezonde controles. In de meeste patiënten met een recidief van de ziekte, daalde de intracellulaire MPO activiteit.

Als laatste hebben we in hoofdstuk 9 de hedendaagse kennis over de rol van MPO in de pathogenese van systeemvasculitis uiteengezet. MPO-ANCA kunnen neutrofielen activeren en laten degranuleren. Hierdoor ontstaat uitgebreide reactieve zuurstof metaboliet formatie en worden actieve enzymen vrijgemaakt, dit leidt tot een versterkte immuunreactie tegen de vaatwand. MPO-ANCA kunnen het verwijderen en inactiveren van MPO door ceruloplasmine tegengaan, wat een verhoogde MPO activiveit veroorzaakt. Actief MPO kan bijdragen aan endotheel dysfunctie en aan de chronische nierafwijkingen van patiënten met MPO-ANCA glomerulonefritis. Verder worden MPO spiegels beïnvloed door hormonale en genetische factoren, zoals het MPO463 en MPO129 promotor polymorfisme. Het MPO463 polymorfisme is geassocieerd met een verhoogd risico op het ontwikkelen van MPO-ANCA geassocieerde ziekte.

Samenvattend speelt MPO een belangrijke rol in vasculitis en arteriosclerose, zowel als maat voor ziekteactiviteit, als onderdeel van het pathogenetisch proces. In de toekomst is het interessant om MPO remmende medicamenten uit te testen als onderdeel van de therapie bij

zowel arteriosclerose als vasculitis. De behandeling van vasculitis bestaat uit de snelle en agressieve immuun modulerende therapie. In arteriosclerose daarentegen, is de meeste therapie gericht op het chronisch management van het ziekteproces. Omdat een gedeelte van de immuunreactie in vasculitis en arteriosclerose overlapt, kan het zeer zinvol zijn om de therapie van deze twee ziekten uit te wisselen.