Methods.—Nasal brushings of the inferior turbinate were obtained from 32 subjects with GPA (n = 10 active nasal disease, n = 13 prior nasal disease, n = 9 never nasal disease) and 35 controls with and without inflammatory nasal disease (n = 12 healthy, n = 15 sarcoidosis, n = 8 allergic rhinitis). Extracted and processed RNA was hybridized to microarrays. Significant gene expression changes associated with GPA versus controls were identified using linear mixed effects models. Functional enrichment of biologic pathways among gene sets was determined using GATHER [1] and GSEA [2]. The relationship of nasal gene expression profiles to previously published peripheral blood gene expression levels associated with GPA [3] was determined using GSEA.

Results.—Expression levels of 1671 genes were associated with GPA (FDR < 0.05). A distinct cluster of genes related to immune response was upregulated in GPA, including SERPINA1 and genes related to pro-inflammatory chemokines and cytokines (P_{GATHER} < 0.0001). Subgroup analyses showed 452 genes associated with active nasal disease, 309 with prior nasal disease, and 0 with never nasal disease in GPA (FDR < 0.1, fold change > 1.5). GSEA revealed complete overlap in the top 20 biologic pathways associated with active and prior nasal disease activity (FDR_{GSEA} < 0.25). Peripheral blood gene expression levels associated with GPA were similarly altered in the nasal gene expression profiles of subjects with active and prior, but not never, nasal disease.

Conclusion.—Transcription signatures from upper airway disease in GPA highlight immune system involvement and are similar between subjects with active and prior nasal disease. Nasal gene expression profiles in GPA may also reflect systemic disease activity.

References

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A24
Both histone methylation and acetylation contribute to the aberrant upregulation of proteinase 3 (PR3) and myeloperoxidase (MPO) genes in patients with ANCA disease
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Introduction.—We investigated whether histone H3k9 methylation and histone acetylation might be disrupted in ANCA patients.

Methods.—Expression levels of H3K9 methyltransferase gene, EHMT1, and H4K16 acetylation gene, MSL1, were determined by Affymetrix microarray (Array) and quantitative PCR (Q-PCR). Di-methyl H3K9 (H3K9me2) and acetylated H4K16 (H4K16ac) levels were measured by chromatin immunoprecipitation (ChiP).

Results.— Array analysis revealed that expression of EHMT1 was significantly reduced (P = 0.03) and MSL1 was elevated (P = 0.03) in leukocytes from ANCA-patients (n = 25) compared to healthy controls (n = 16). The expression levels of EHMT1 negatively and MSL1 positively correlated with PR3 and MPO mRNA level, which were markedly elevated in ANCA-patients. Expression of EHMT1 was significantly lower in MPO-ANCA (n = 12) than PR3-ANCA patients (n = 13) and MSL1 was higher in PR3-ANCA than MPO-ANCA patients. Q-PCR confirmed the reduced expression of EHMT1 (P = 0.0001) and the increased expression of MSL1 (P = 0.0005) in patients (n = 80) compared to controls (n = 20), and the negative correlations for EHMT1 and the positive correlations for MSL1 compared to PR3 and MPO genes. EHMT1 mRNA level was significantly lower (P = 0.01) and MSL1 higher (P = 0.0003) in patients with active disease (n = 40) than patients in remission (n = 40).

ChiP showed that H3K9me2 was statistically depleted at the PR3 and MPO promoter regions in MPO-ANCA patients (n = 7) compared to controls (n = 23; P = 0.01), but not in PR3-ANCA patients (n = 8). ChiP also showed that H4K16ac was increased at the PR3 and MPO promoter regions in half of ANCA patients (n = 10) compared to controls (n = 10), with the highest H4K16ac levels seen in PR3-ANCA patients with the highest PR3 expression.

Discussion.—These data further implicate epigenetic mechanisms in the regulation of ANCA autoantigen genes.

Conclusion.—The epigenetic modifications at PR3 and MPO genes suggest that transcriptional control of autoantigen genes differs in MPO-ANCA versus PR3-ANCA patients.

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A25
Reclassifying ANCA-associated vasculitis using the EUVAS algorithm: Epidemiology and survival from the NORVASC register
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Introduction.—GPA and MPA are traditionally combined under the term ANCA-associated vasculitis (AAV). Mahr using the EUVAS trial cohorts. The proposed algorithm uses clinical features and PR3-ANCA presence to classify AAV into five subgroups: cardiovascular (CV AAV), gastrointestinal (GI AAV), non-renal AAV, Renal AAV with PR3-ANCA and renal AAV without PR3-ANCA. These groups have distinct mortality and relapse outcomes. We aimed to see if the distribution of patients and mortality pattern was the same in a population-based cohort as in the trial cohorts.

Methods.— We reviewed the prospective Norfolk vasculitis Register (NORVASC) for cases of GPA and MPA diagnosed between 2000 and 2010. We applied the Mahr algorithm to reclassify patients into the five subgroups. We calculated incidence and 95% CI using the Poisson dis-
distribution. Survival characteristics were calculated using the Kaplan-Meier method.

**Results.**—Between 2000 and 2010, 82 patients were diagnosed with GPA or MPA (F/M:44/38). Cases were classified into the five subgroups (Table I). Compared with the EUVAS patients CV AAV was less frequent and GI AAV more frequent. The two types of renal AAV were of comparable frequency. The overall incidence of AAV was 16.2/1,000,000 (12.9–20.2). The median follow-up was 5.36 years (0.05–12.2 y). The 2 year survival was worst in the GI AAV group (70%) and best in the renal PR3 group.

**Discussion.**—In our cohort, the five subgroups of AAV have distinct survival curves supporting the idea that subcategorization of AAV would be useful in predicting outcome. The proportion of patients classified into each group is similar to the EUVAS trial. We have confirmed in an unselected population that PR3+ve renal vasculitis has a better outcome than renal vasculitis without PR3 and also that GI AAV is associated with a worse outcome.

**Conclusion.**—We have confirmed using the Mahr algorithm in an unselected cohort results in a similar classification to the trial patients.

**Reference**

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**A27 ENT involvement is related to better renal function in patients with ANCA-associated vasculitis**


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13. Department of Internal Medicine, Meander Medical Center, Amersfoort, Netherlands

Patients were diagnosed with GPA or MPA

<table>
<thead>
<tr>
<th>N (%)</th>
<th>Incidence (/1000000 pop)</th>
<th>1 y survival (%)</th>
<th>2 y survival (%)</th>
<th>5 y survival (%)</th>
<th>10 y survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CV AAV</td>
<td>3(3.7)</td>
<td>0.6 (0.1–0.7)</td>
<td>100</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>GI AAV</td>
<td>10(12.2)</td>
<td>2.0 (1.0–3.6)</td>
<td>70</td>
<td>70</td>
<td>58</td>
</tr>
<tr>
<td>Non-renal AAV</td>
<td>12(14.6)</td>
<td>2.4 (1.4–4.2)</td>
<td>100</td>
<td>83</td>
<td>73</td>
</tr>
<tr>
<td>Renal AAV +PR3</td>
<td>30 (36.5)</td>
<td>5.9 (4.0–8.5)</td>
<td>100</td>
<td>100</td>
<td>96</td>
</tr>
<tr>
<td>Renal AAV —PR3</td>
<td>27 (32.9)</td>
<td>5.3 (3.5–7.8)</td>
<td>93</td>
<td>85</td>
<td>71</td>
</tr>
</tbody>
</table>

http://dx.doi.org/10.1016/j.lpm.2013.02.028

**A26 A myelopoiesis gene signature during remission in ANCA-associated vasculitis reflects ongoing prednisolone therapy and does not seem to predict relapses**


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**Introduction.**—A myelopoiesis gene signature in circulating leucocytes, exemplified by increased mRNA levels for MPO and PR3, has been reported in ANCA-associated vasculitis (AAV), possibly related to disease activity. We explored its relation to subsequent relapses, treatment, selected intracellular transcription factors and microRNAs (miRs).

**Patients.**—RNA was isolated from peripheral blood mononuclear cells (PBMCs) and polymorphonuclear cells (PMNs) in 67 AAV patients (45 MPA, 22 GPA) and 27 controls. mRNA for PR3, MPO, transcription factors and miRs were analyzed with Taqman qPCR. Patients were followed prospectively for 10–23 months.

**Results.**—Patients had higher mRNA levels in PBMCs for MPO and PR3, and in PMNs for PR3. Patients with active disease (n = 6) tended to have further elevated levels. 11 patients developed relapses during follow-up; their mRNA levels were not elevated compared to those who remained in remission. mRNA levels did not differ based on treatment with Azathioprine, Mycophenolate or Methotrexate, but correlated to steroid doses. Steroid-free AAV patients (n = 16) had levels similar to controls. In controls mRNA levels for MPO and PR3 were correlated to C/EBP-α in PBMCs but such a correlation was not seen in AAV patients. In controls both PR3 and MPO mRNA levels correlated to miR-29a, -93 and -142-3p. In AAV patients there were no significant correlations between PBMC miR levels and mRNA for MPO/PR3. Also in PMNs from controls there were several positive correlations between miRs and MPO/PR3 mRNA that were not present in PMNs from AAV patients. **Conclusion.**—The regulation of MPO and PR3 mRNA levels seems to differ between AAV patients and controls. Low doses of steroids (2.5–10 mg/day) might be responsible for these differences as well as for the myelopoiesis gene signature seen in AAV during remission. mRNA levels for PR3 and MPO do not seem to reflect subclinical disease activity or predict relapses. If these findings relate to the therapeutic effect of steroids in AAV remains unknown.

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