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Review

The value of anti-neutrophil cytoplasmic antibodies (ANCA) testing for the diagnosis of ANCA-associated vasculitis, a systematic review and meta-analysis

Niels A.D. Guchelaar [a], Manon M. Waling [a], Anviti A. Adhin [a], Paul L.A. van Daele [a], Marco W. J. Schreurs [b], Saskia M. Rombach [a,*]

[a] Department of Internal Medicine, Allergy and Clinical Immunology, Erasmus MC University Medical Center, Rotterdam, the Netherlands
[b] Department of Immunology, Laboratory Medical Immunology, Erasmus MC University Medical Center, Rotterdam, the Netherlands

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ABSTRACT

The testing of anti-neutrophil cytoplasmic antibodies (ANCA) takes an important place in the diagnostic workup to ANCA-associated vasculitis (AAV). Nowadays, it is recommended to screen for the presence of PR3 and MPO specific antibodies first using immunoassay, without the need for ANCA measurement by indirect immunofluorescence (IIF). A literature search was performed to assess the diagnostic test value of ANCA IIF and PR3- and MPO-antibody immunoassay to diagnose AAV. This meta-analysis shows that the c-ANCA testing by IIF has a pooled sensitivity of 75.2% and a pooled specificity of 98.4%. For PR3-antibody immunoassay, the pooled sensitivity depended on the immunoassay method used, and ranged from 79.8% to 86.6%, whereas the pooled specificity ranged from 96.8% to 98.3%. For both p-ANCA IIF and MPO-antibody immunoassay (all methods) sensitivity varied considerably showing pooled values of respectively 46.3% and 58.1%, whereas respective pooled specificity was 91.4% and 95.6%. These findings support the 2017 international consensus that primary anti-PR3 and anti-MPO screening by immunoassay, based on superior immunoassay sensitivity without the need for IIF ANCA testing, improves the diagnostic workup of AAV.

1. Introduction

Antineutrophil cytoplasmic antibodies (ANCA) are antibodies that attack cytoplasmic components of neutrophils and monocytes [1]. These antibodies are of major importance in detecting and classifying systemic vasculitides [2], such as granulomatosis with polyangiitis (GPA, previously named Wegener granulomatosis (WG)) [3], microscopic polyangiitis (MPA) [4] and eosinophilic granulomatosis with polyangiitis (eGPA, previously named Churg-Strauss Syndrome (CSS)) [5]. An international consensus regarding the classification of ANCA-associated vasculitides is currently in use, namely the Chapel Hill Consensus [6]. GPA, MPA and eGPA are called ANCA-associated vasculitis (AAV) because of their similar clinical and pathological features and treatment options. AAV affects mostly the small and medium-sized blood vessels, and ANCA tests are usually positive [1,7]. These vasculitides can have different systemic manifestations which may damage vital organs and consequently lead to serious morbidity and mortality [1].

ANCA measurement can be used as a screening tool in clinical practice [8]. These measurements are commonly used as screening for AAV. AAV manifests itself in different ways and comes with a variety of symptoms. Importantly, clinicians should use ANCA screening only when sufficient clinical suspicion is present because it can lead to false-positive results [9,10]. Besides that, the prevalence of AAV is low with a reported number of 42/100,000 in the United States [11]. Therefore, some cautiousness in requesting ANCA-testing is needed.

There are two forms of ANCA testing, namely the indirect immunofluorescence assay (IIF) and the immunoassay, such as enzyme-linked immunosorbent assay (ELISA) [12,13]. The IIF test is performed with ethanol-fixed human neutrophils as substrate and hereby there are two main fluorescence patterns visible. ANCA can display a granular cytoplasmic pattern (c-ANCA) or a perinuclear pattern (p-ANCA) [9]. Cytoplasmic ANCA (c-ANCA) aimed towards proteinase 3 (PR3-ANCA) [14,15] is usually positive in patients with GPA and perinuclear ANCA (p-ANCA) aimed towards myeloperoxidase (MPO-ANCA) [4] often appears in patients suffering from MPA or eGPA.

However, not all patients with systemic vasculitis are ANCA IIF

* Corresponding author.
E-mail address: s.rombach@erasmusmc.nl (S.M. Rombach).

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positive so ANCA testing with IIF may not be sensitive enough [8]. Furthermore, ANCAs are not specific for vasculitis because immunofluorescence testing can also detect (atypical) ANCA patterns in other inflammatory diseases [8,16] like drug-induced vasculitis, ulcerative colitis, primary sclerosing cholangitis, autoimmune hepatitis, rheumatoid arthritis, SLE, interstitial lung disease, cystic fibrosis and infections. The ELISA based immunoassay makes use of purified specific antigens. Immunoassays detecting antibodies against PR3 and MPO usually have higher specificities and positive predictive values compared to IIF patterns like p-ANCA and c-ANCA [17,18]. Hence, it was suggested to perform an immunoassay in combination with IIF [8,19].

There are three types of antibody immunoassay methods, based on their antibody detection, namely direct, capture and anchor immunoassay. Respectively, these tests are also called the first-generation, second-generation and third-generation assays. In direct immunoassay, the carrier is directly coated with the antigen. In capture immunoassay, the carrier is coated with specific monoclonal antibodies that capture and present the antigen. Finally, in anchor immunoassay the antigen is bound to the carrier with a peptide linker [20]. Several antibody detection test systems are available that employ the above mentioned immunoassays, such as enzyme-linked immunosorbent assay (ELISA), fluorescent enzyme immunoassay (FEIA) and chemiluminescent immunoassay (CLIA).

In the 1999 international consensus statement, ANCA screening by indirect immunofluorescence (IIF) was recommended in suspected AAV and in case of ANCA IIF positivity, specific detection of PR3 and MPO antibodies was advised [21]. Although this testing algorithm is still widely used, its diagnostic performance has recently been questioned. A more recent multicenter study showed that the diagnostic performance of current antigen-specific immunoassays equals or even exceeds the diagnostic performance of ANCA IIF to discriminate AAV from disease controls [22]. These findings resulted in a new international consensus. This 2017 international consensus on ANCA testing advised that immunoassay should be used as a primary screening method for diagnosing AAV, without the need for IIF [23]. Though data on the diagnostic test value of both IIF and immunoassay ANCA testing are reported by different research groups, a meta-analysis of the data has not yet been performed. This analysis could be of value in the evaluation of the new consensus. Therefore, we performed a systematic review and meta-analysis to evaluate the diagnostic value of ANCA testing using IIF and immunoassay (thereby comparing direct, capture and anchor immunoassay) in patients with AAV.

2. Methods

2.1. Search strategy

For this systematic review and meta-analysis, a literature search was performed using the Medline (PubMed) database and finalized on the 16th of September 2019. The search terms were: ("Anti-Neutrophil Cytoplasmic Antibody-Associated Vasculitis"[Mesh] AND "Diagnosis, Differential"[Mesh] AND "Antibodies, Antineutrophil Cytoplasmic"[Mesh]) OR ("Sensitivity and Specificity"[Mesh] AND "Antibodies, Antineutrophil Cytoplasmic"[Mesh]) OR ("ANCA"[tiab] AND "Anti-Neutrophil Cytoplasmic Antibody-Associated Vasculitis"[tiab] AND "diagnosis"[tiab]). An additional search was performed without Mesh terms to be able to identify the most recent publications.

2.2. Selection criteria

For this systematic review, eligible studies met the following inclusion criteria: Investigation of the diagnostic value of cytoplasmic antineutrophil cytoplasmic antibodies (c-ANCA), perinuclear antineutrophil cytoplasmic antibodies (p-ANCA), proteinase 3 (PR3) and/or myeloperoxidase (MPO) antibodies in the blood samples from patients with AAV; The sensitivity, specificity and sample size or positive predictive value, negative predictive value and sample size were provided; Measurements were done using indirect immunofluorescence and/or immunoassay. We included all forms of immunoassay, such as ELISA, FEIA and CLIA. Studies that only included immunofluorescence and not immunoassay were excluded. Letters, reviews and case reports were excluded.

The study selection process was done by three researchers independently (NG, MW and AA). First, titles and abstracts were screened based on relevance. The studies that did not address the research question were excluded. Eventually, the full-text articles were screened and a definite set of studies was determined. Disagreements were resolved by consensus.

2.3. Data extraction and quality assessment

Data extraction was performed by the three researchers independently. We extracted the name of the first author, year of publication, type of study, sample size, number of patients and controls and diagnostic test values (sensitivity, specificity, positive predictive value and negative predictive value, or calculated these based on the reported patients numbers and test positivity).

We used the Quality Assessment Tool for Diagnostic Accuracy Studies (QUADAS-2 tool) [24] to assess the quality of the articles, as advised by the Cochrane guidelines [25]. This tool evaluates each study on four domains, namely patient selection, index test, reference standard and timing. These domains are appraised using 14 questions, as described in Appendix A. A study is classified as low risk of bias when it has a maximum of 1 negative point in each section [24].

2.4. Statistical analysis

A meta-analysis was done to estimate the sensitivity and specificity of c-ANCA tested by indirect immunofluorescence and for the sensitivity and specificity of anti-PR3 tested by immunoassay. The analysis for immunoassay was done separately for direct immunoassay, capture immunoassay and anchor immunoassay. For p-ANCA and anti-MPO a separate meta-analysis was performed. We used the true positives, false positives, false negatives and true negatives that we determined using the reported sensitivity, specificity, disease group and control group. The forest plot and summary receiver operating characteristics (SROC) plot were constructed using Review Manager Software 5.3 empowered by Cochrane Library [26]. Summary sensitivity and specificity were calculated with the bivariate model using the MetaDTA tool [27]. We derived 95% CI's for all estimated values, including the summary measures. We also calculated the likelihood ratios using the sensitivity and specificity for each test by using the formulas: LR+ = sensitivity / (1 – specificity) and LR- = specificity / (1 – sensitivity) [28].

3. Results

3.1. Literature search

The literature search resulted in a total of 721 unique articles. The screening of the articles by title and abstract led to an exclusion of 656 articles. The remaining 65 articles were fully analyzed for eligibility using the inclusion and exclusion criteria. This resulted in an exclusion of 52 articles (Fig. 1). Therefore, 13 articles finally met the inclusion criteria and were included in this meta-analysis. Fig. 1 shows the detailed selection process.

3.2. Study characteristics

The characteristics of the included studies are shown in Table 1. Studies were published from 1998 to 2017 and the number of subjects varied between 78 and 4620. Thereafter, there were no new eligible studies published between 2017 and 2019. All the articles except one

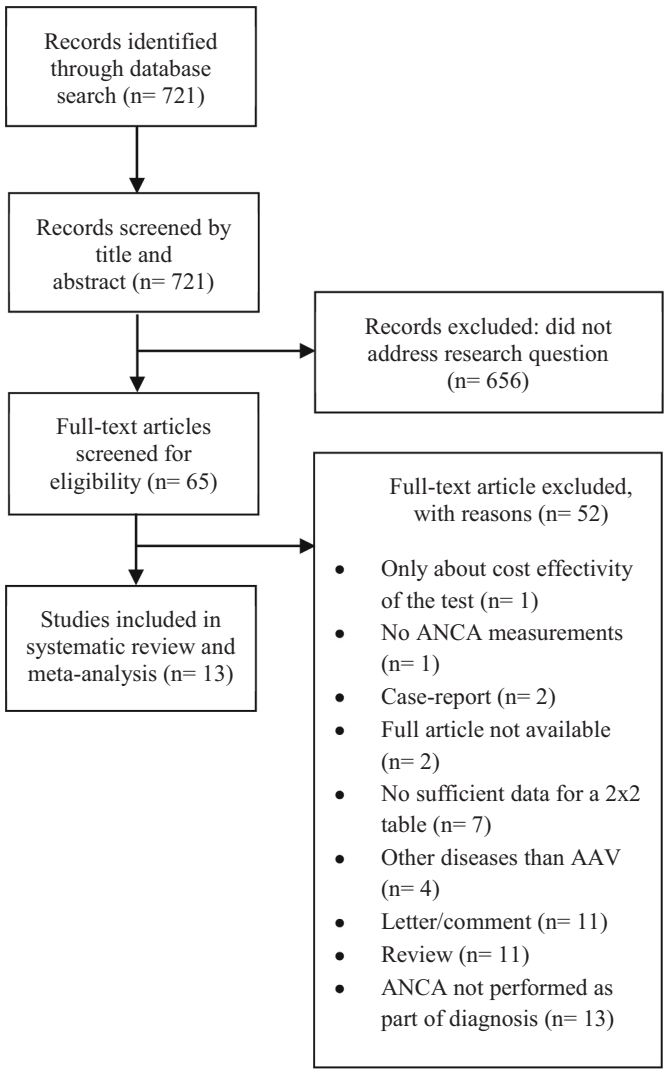

Fig. 1. Flowchart of the study selection.

were published in English. This article was written in Norwegian [29] and was translated into English to be able to extract the data. Diseases that were included in the studies as part of the ANCA-associated vasculitides (AAV) were granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA), eosinophilic granulomatosis with polyangiitis (eGPA), renal limited vasculitis (RLV), idiopathic rapidly progressive glomerulonephritis (iRPGN) and pauci-immune glomerulonephritis (PIGN). The diagnosis was made based on the clinical features in all studies, according to the Chapel Hill Consensus [6]. ANCA testing was done with immunoassay and in some studies also with IIF. A total of 9 out of 13 studies included sensitivity and specificity of c-ANCA. 12 out of 13 studies reported sensitivity and specificity of anti-PR3. Only 4 out of 13 studies examined sensitivity and specificity of p-ANCA and 5 out of 13 studies anti-MPO.

We used the QUADAS-2 tool to assess the quality of the articles. All studies had a potential risk of bias, when the QUADAS-2 tool was used. Appendix A shows a detailed assessment of each study.

3.3. c-ANCA and anti-PR3 test value

There were 9 studies that reported the sensitivity and specificity for c-ANCA IIF. These studies including their sensitivity and specificity with 95% CI are shown in Fig. 2. The pooled sensitivity estimate of c-ANCA is 75.2% (95% CI, 60.7% to 85.6%) and the pooled specificity estimate is 98.4% (95% CI, 92.8% to 99.7%), as shown in the SROC curve (Fig. 3).

A total of 12 studies described the sensitivity and specificity of anti-PR3 immunoassay. 10 studies used direct immunoassay as the method of immunoassay. An overview of these studies, as well as their sensitivity and specificity with 95% CI, is presented in Fig. 4. The pooled sensitivity estimate of anti-PR3 by direct immunoassay is 79.8% (95% CI, 59.0% to 91.9%) and the pooled specificity estimate is 98.3% (95% CI, 93.9% to 99.5%). This is also illustrated in the SROC curve (Fig. 5). Four studies used capture immunoassay with a pooled sensitivity of 84.9% (95% CI, 71.0% to 92.8%) and a specificity of 98.8% (95% CI, 97.2% to 99.5%) for anti-PR3 [30,32,34,36]. The forest plot and SROC curve for anti-PR3 using capture immunoassay is visible in Appendix B. Five studies used anchor immunoassay for determining anti-PR3 [22,30,32,33,36]. The pooled sensitivity for anchor immunoassay is 86.6% (95% CI, 74.8% to 93.3%) and the pooled specificity is 96.8% (95% CI, 91.8% to 98.8%) (Appendix C).

3.4. p-ANCA and anti-MPO test value

A few studies investigated the p-ANCA and anti-MPO test value. There were 4 studies in total that studied sensitivity and specificity for p-ANCA tested by IIF. The pooled sensitivity for p-ANCA is 46.3% (95% CI, 14.4% to 81.6%) and the pooled specificity is 91.4% (95% CI, 80.8% to 96.4%). The forest plot and SROC curve are presented in respectively Figs. 6 and 7.

The anti-MPO immunoassay was examined in 5 studies. The pooled sensitivity for anti-MPO is 58.1% (95% CI, 34.6% to 78.5%) and the specificity is 95.6% (95% CI, 92.0% to 97.6%) (Appendix D). All studies used direct immunoassay, except for Damoiseaux et al. [22] that used anchor immunoassay. For this reason, we did not perform separate analyses for the different anti-MPO immunoassay methods.

Noel et al. [35] used three types of direct ELISA testing. Therefore, we used the results of test A because this was the standard method. Radice et al. [36] used different tests for each assay, we chose to use the best performing test for direct ELISA, capture ELISA and anchor ELISA. Hagen et al. [13] described sensitivity and specificity for the different forms of AAV individually. We pooled the sensitivities and specificities to calculate a weighted average, using the formula for a weighted average as shown in Appendix E. Because of this, we could include the study in our meta-analysis.

3.5. Subanalysis: eGPA

The 2017 consensus on ANCA testing [23] is applicable for GPA and MPA only. We performed a subanalysis to assess if the sensitivity and specificity significantly differed if only studies including eGPA patients were included in the meta-analysis. 8 out of 13 studies [10,13,17,30,31,33,34,37] included eGPA patients. Repeating the meta-analysis, including those eight studies, sensitivity and specificity were comparable (data not shown).

3.6. Likelihood ratios

We have calculated the positive likelihood ratio (LR+) and negative likelihood ratio (LR-) of each diagnostic test using the pooled sensitivities and specificities. A likelihood ratio is used to estimate whether a test result changes the probability that an ANCA-associated vasculitis exists. For c-ANCA IIF, a LR+ of 47 and a LR- of 3.97 was calculated. Anti-PR3 by direct immunoassay gives a LR+ of 47 and a LR- of 4.87. For capture immunoassay anti-PR3 the LR+ is 71 and the LR- 6.54. A LR+ of 27 was calculated for anchor immunoassay anti-PR3 alongside of a LR- of 7.22. For p-ANCA (LR+ 5.38 and LR- 1.70) and anti-MPO (LR+ 13 and LR- 2.28) the likelihood ratios were also calculated.

Table 1
Study characteristics.

Authors	Year	Journal	Study design	Number of controls	Disease(s)	Confirmed diagnosis	Method of testing
Damoiseaux et al. [22]	2017	Ann Rheum Dis	Comparative, multicenter, evaluation study	924	GPA, MPA	251	IIF, immunoassay (anchor)
De Joode et al. [30]	2014	Eur J Intern Med	Retrospective, cohort study	198	GPA, MPA, RLV, eGPA	62	IIF, immunoassay (anchor and capture)
Hagemo et al. [29]	2002	Tidsskr Nor Laegeforen	Comparative study	292	GPA	27	IIF, immunoassay (direct)
Hagen et al. [13]	1998	Kidney Int	Multicenter, case-control study	924	GPA, MPA, eGPA, iRPGN	275	IIF, immunoassay (direct)
Harris et al. [31]	1999	J Clin Pathol	Prospective, clinical trial	344	GPA, MPA, polyarteritis nodosa, eGPA	43	IIF, immunoassay (direct)
Hellmich et al. [32]	2007	Clin Exp Rheumatol	Prospective, clinical trial	530	GPA	86	IIF, immunoassay (direct, anchor and capture)
Houben et al. [33]	2016	Medicine (Baltimore)	Retrospective, cohort study	118	MPA, GPA, eGPA	119	Immunoassay (anchor)
Ito-Ihara et al. [34]	2008	Clin Exp Rheumatol	Comparative study	109	GPA, MPA, eGPA	64	Immunoassay (direct and capture)
Noel et al. [35]	2013	Autoimmun Rev	Evaluation study	59	GPA	19	Immunoassay (direct)
Phatak et al. [10]	2017	Int J Rheum Dis	Retrospective study	1563	GPA, MPA, eGPA, PIGN	27	IIF, immunoassay (direct)
Radice et al. [36]	2013	Clin Chem Lab Med	Comparative study	195	GPA	55	IIF, immunoassay (direct, anchor and capture)
Schönermarck et al. [37]	2001	Rheumatology (Oxford)	Comparative study	4236	GPA, eGPA, MPA	384	IIF, immunoassay (direct)
Stone et al. [17]	2000	Arthritis Care Res	Cross-sectional study	787	GPA, eGPA, MPA, PIGN	69	IIF, immunoassay (direct)

Abbreviations: *GPA*: Granulomatosis with Polyangiitis; *MPA*: Microscopic Polyangiitis; *eGPA*: Eosinophilic Granulomatosis with Polyangiitis; *RLV*: Renal Limited Vasculitis; *iRPGN*: Idiopathic Rapidly Progressive Glomerulonephritis; *PIGN*: Pauci-immune Glomerulonephritis; *IIF:* Indirect Immunofluorescence.

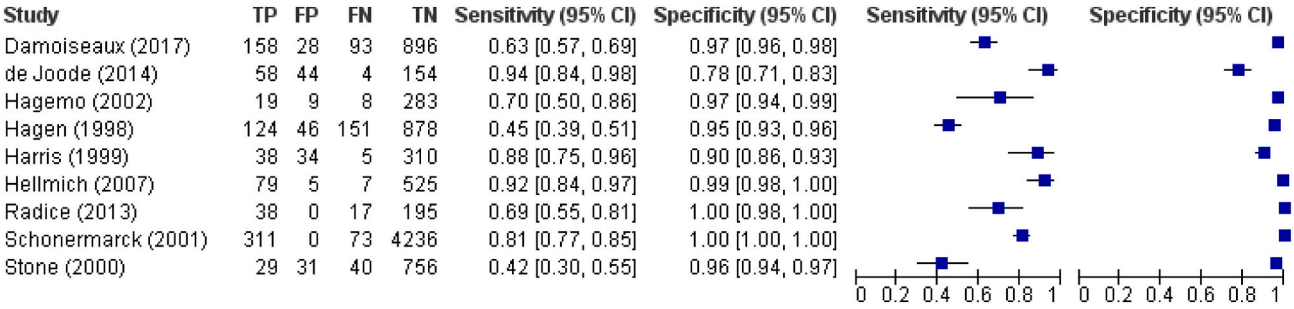

Fig. 2. Forest plot: sensitivity and specificity for c-ANCA. TP (True Positives), FP (False Positives), FN (False Negatives) and TN (True Negatives) were calculated using the reported sensitivity, specificity, total subjects and positive tests.

4. Discussion

The only other available meta-analysis so far with a pooled sensitivity and specificity for c-ANCA in a large group of patients is a review from Rao et al. from 1995, when measurement of anti-PR3 and anti-MPO was not yet clinical practice [38]. Sensitivity and specificity for c-ANCA in the previous study were comparable to the sensitivity and specificity in current meta-analysis. This meta-analysis shows the measurement of anti-PR3 and anti-MPO is of additional value, especially because of its high specificity.

The recent 2017 international consensus [23] proposes that immunoassay can be used as a screening method for patients suspected of having ANCA-associated vasculitis, especially GPA and MPA, without ANCA screening with IIF first. This was based on the results of a multicenter European Vasculitis Study Group (EUVAS) evaluation [22,39]. The number of patients included in this evaluation was limited, however (GPA = 186, MPA = 65) [22]. This meta-analysis endorses the 2017 consensus that PR3- and MPO-antibody immunoassay is the most reliable screening method for AAV. The consensus is currently only in use for GPA and MPA, although eGPA patients were included in 8/13 studies. Repeating the analysis with the 8 studies including eGPA patients, sensitivity and specificity were comparable. However, this is probably explained by the fact that in most studies the eGPA patients were a minority of the included patients. Sensitivity and specificity of ANCA testing by IIF and immunoassay should be determined in a large cohort of eGPA patients only, to investigate this further.

The pooled sensitivity for c-ANCA was 75.2% and for anti-PR3 was 79.8% in case of direct immunoassay. For capture immunoassay the pooled sensitivity was 84.9% and for anchor immunoassay 86.6%. The 95% confidence intervals for the sensitivity are wide. This raises the question if a positive ANCA test could be used as a suitable confirmation for the diagnosis of a suspected AAV. Ideally, a biopsy would prove the diagnosis of AAV [40]. However, in clinical practice, a biopsy is not always done and the diagnosis of AAV is based on both the clinical features and ANCA serology [41–43]. In this meta-analysis a combination of a positive c-ANCA and anti-PR3 was mostly associated with a diagnosis of granulomatosis with polyangiitis. Furthermore, a positive p-ANCA and anti-MPO was mainly related to microscopic polyangiitis. Alternative diagnoses that counted for a large amount of ANCA positive patients were inflammatory bowel disease, rheumatoid arthritis, an infection, such as human immunodeficiency virus, and connective tissue diseases. These patients were more often positive for c-ANCA or p-ANCA than for anti-PR3 or anti-MPO. Other reported diagnoses were drug-induced vasculitis, including cocaine-induced vasculitis [17,35] and are usually p-ANCA positive in combination with positive anti-MPO and/or positive anti-PR3. A positive anti-elastase test would also be present in most cases of drug-induced vasculitis [44]. When the ANCA IIF is negative, but the result of anti-MPO/PR3-testing is positive, there

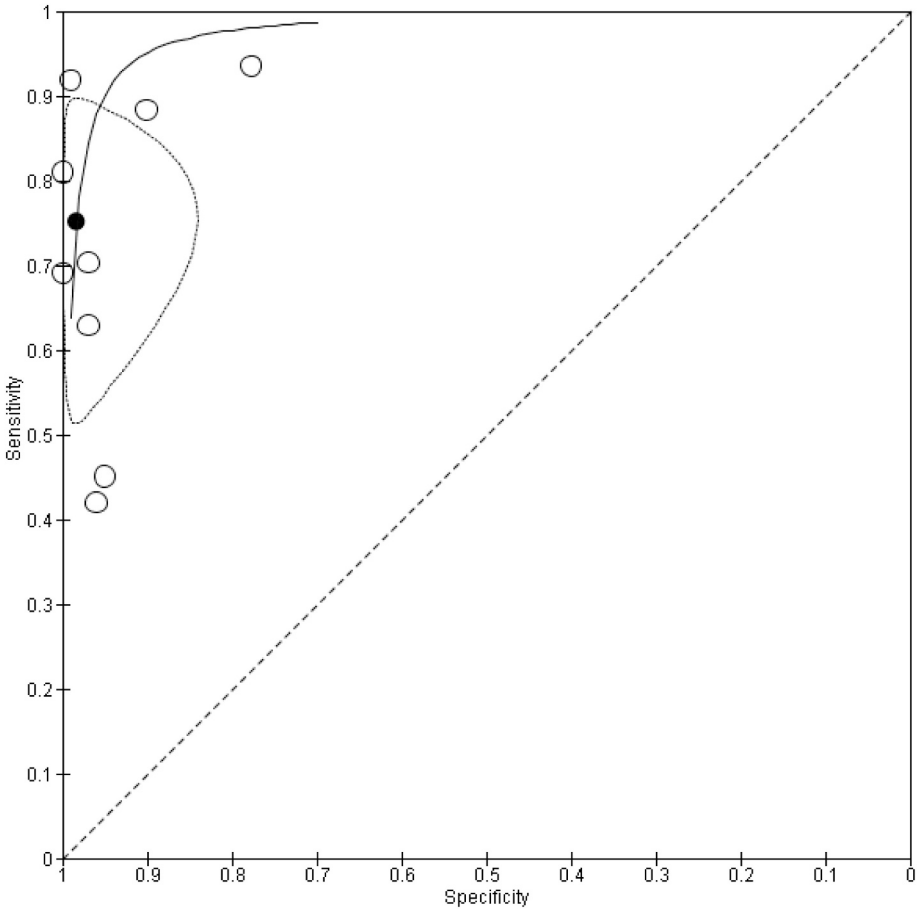

Fig. 3. SROC curve for c-ANCA with pooled sensitivity (75.2%) and specificity (98.4%) with 95% CI.

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Hagemo (2002)	17	3	10	289	0.63 [0.42, 0.81]	0.99 [0.97, 1.00]
Hagen (1998)	141	118	134	806	0.51 [0.45, 0.57]	0.87 [0.85, 0.89]
Harris (1999)	37	10	6	334	0.86 [0.72, 0.95]	0.97 [0.95, 0.99]
Hellmich (2007)	52	5	34	525	0.60 [0.49, 0.71]	0.99 [0.98, 1.00]
Ito-Ihara (2008)	64	2	0	107	1.00 [0.94, 1.00]	0.98 [0.94, 1.00]
Noel (2013)	19	23	0	36	1.00 [0.82, 1.00]	0.61 [0.47, 0.73]
Phatak (2017)	23	16	4	1547	0.85 [0.66, 0.96]	0.99 [0.98, 0.99]
Radice (2013)	40	9	15	186	0.73 [0.59, 0.84]	0.95 [0.91, 0.98]
Schonermarck (2001)	265	0	119	4236	0.69 [0.64, 0.74]	1.00 [1.00, 1.00]
Stone (2000)	24	8	45	779	0.35 [0.24, 0.47]	0.99 [0.98, 1.00]

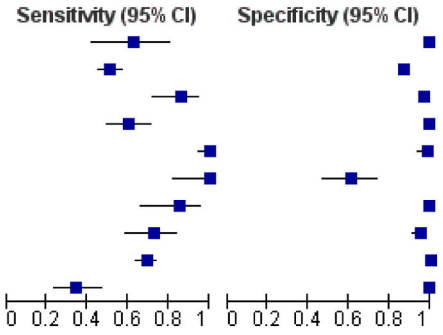

Fig. 4. Forest plot: sensitivity and specificity for anti-PR3 direct immunoassay. TP (True Positives), FP (False Positives), FN (False Negatives) and TN (True Negatives) were calculated using the reported sensitivity, specificity, total subjects and positive tests.

may be a GPA present limited only to the upper respiratory tract. However, a study with IF-negative and anti-MPO or -PR3 positive results also revealed diagnoses of non-vasculitis conditions, mainly IBD and SLE [45].

Studies are not consistent in reporting the most common alternative diseases with dual positivity for PR3 and MPO antibodies. One study found that also a malignancy or a recurrent infection could be the cause of a dual positivity [46]. A clinician should also be aware of drug-induced vasculitis with dual positivity. ANCA-positivity is shown to be common with anti-thyroid medication, especially propylthiouracil [46,47]. The prevalence of AAV is rare, with a reported prevalence in the United States of 42/100,000 [11]. In a non-specialistic setting, the value of a positive ANCA test is low because of its low pre-test probability. However, in a specialistic setting, a positive ANCA test has a higher probability of being a true ANCA-associated vasculitis.

We found high specificities for both c-ANCA (98.4%) and anti-PR3 (direct immunoassay 98.3%, capture immunoassay 98.8% and anchor immunoassay 96.8%). This indicates that a negative ANCA test, especially in a clinical specialistic setting, could be of great value for excluding an AAV. A specificity of 100% is practically not possible in a diagnostic test, especially in biological markers such as ANCA as they slightly differ between patients. There are several known epitopes involved in the ANCAs and therefore the binding in an immunoassay could differ between patients [48].

Most commonly, ELISA was used as the test system of immunoassay. Direct, capture and anchor immunoassay showed a comparable sensitivity and specificity (95% CI overlap) for detection of anti-PR3. All types of immunoassay showed a comparable specificity of almost 100%.

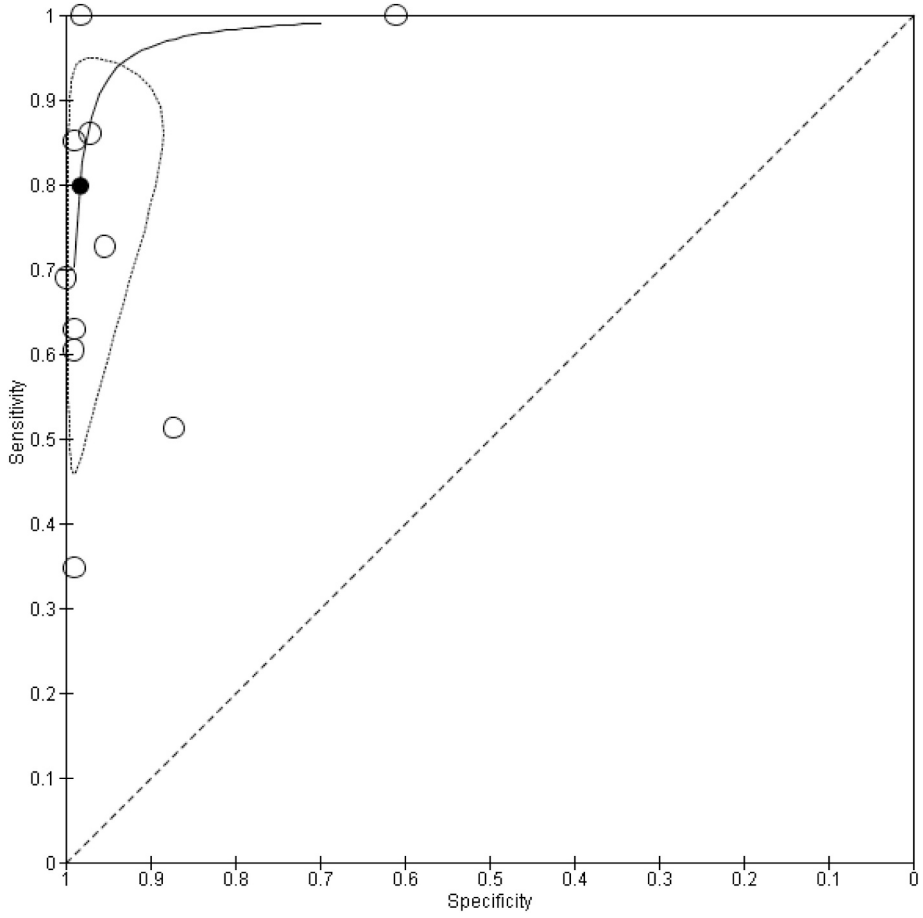

Fig. 5. SROC curve for anti-PR3 direct immunoassay with pooled sensitivity (79.8%) and specificity (98.3%) with 95% CI.

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Damoiseaux (2017)	822	48	102	203	0.89 [0.87, 0.91]	0.81 [0.75, 0.86]
Hagemo (2002)	2	9	25	283	0.07 [0.01, 0.24]	0.97 [0.94, 0.99]
Hagen (1998)	95	176	180	748	0.35 [0.29, 0.40]	0.81 [0.78, 0.83]
Schonermarck (2001)	250	254	134	3982	0.65 [0.60, 0.70]	0.94 [0.93, 0.95]

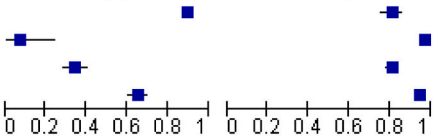

Fig. 6. Forest plot: sensitivity and specificity for p-ANCA. TP (True Positives), FP (False Positives), FN (False Negatives) and TN (True Negatives) were calculated using the reported sensitivity, specificity, total subjects and positive tests.

We have also calculated the likelihood ratios for each test using the pooled sensitivity and specificity [49]. A LR+ of more than 10 is associated with a clinical significant result [28]. In this study, the LR+ was above 10 for both anti-PR3 and anti-MPO. This means, when an ANCA-associated vasculitis is expected, a positive test result increases the chances of an ANCA-associated vasculitis even more. The other way around, the LR- was not below 1. This means a negative test result for anti-PR3 or anti-MPO, does not exclude an ANCA-associated vasculitis.

This review has several limitations. The studies included had different purposes, characteristics, study designs, methods of testing, observed diseases and patient populations. Most studies were comparative [13,22,29,34,36,37], some were prospective cohort studies [31,32,35] and some retrospective cohort studies [10,30,33]. The study by Stone et al. [17] was a cross-sectional study. Although different study designs were used in the included studies, we do not think that this substantially influenced the results. A diagnosis of AAV was namely confirmed according to the guidelines in all included studies.

All studies included diseases that belong to the group of ANCA-associated vasculitides. GPA was the disease that was included in most studies. Therefore, the sensitivity and specificity of c-ANCA and anti-PR3 are mostly applicable on diagnosing GPA.

We also performed a meta-analysis on the sensitivity of p-ANCA and anti-MPO. The pooled sensitivity was fairly low for both p-ANCA (46.3%) and anti-MPO (58.1%) and the confidence intervals were wide. This could be explained by the fact that the studies mostly included patients with GPA, and not with MPA. MPA is mostly associated with a positive p-ANCA and anti-MPO, as opposed to GPA. The specificity however, was high for both p-ANCA (91.4%) and anti-MPO (95.6%).

A possible limitation is that two articles were not included, because the full article was not available. We contacted the authors; however, no reply was given. Additionally, it would also have been valuable to investigate studies that presented sensitivity and specificity when a combination of immunofluorescence and immunoassay was used. Most studies did not meet this criteria, however, although Stone et al. showed the combination of both IF and immunoassay increases both the positive and negative predictive value [17].

A quality assessment was performed. For systematic reviews on diagnostic test accuracy, the QUADAS-2 tool is advised [25]. This tool assesses each study on four domains, namely patient selection, index test, reference standard and flow/timing, using 14 predetermined

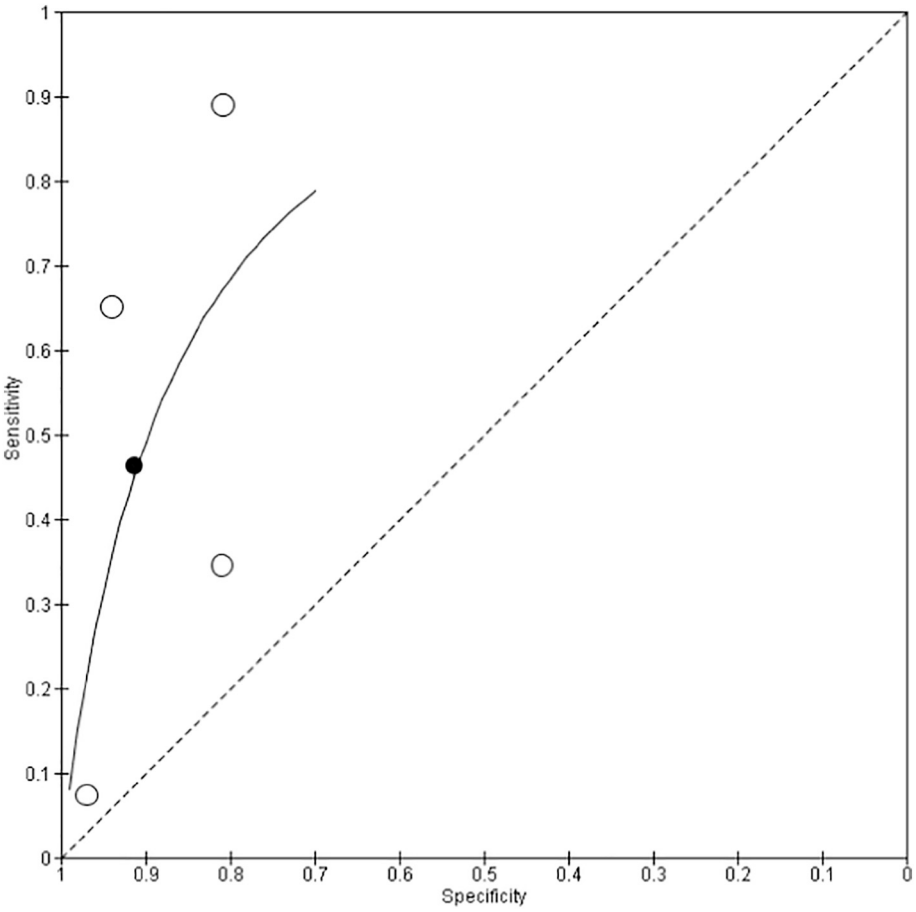

Fig. 7. SROC curve for p-ANCA with pooled sensitivity (46.3%) and specificity (91.4%) with 95% CI.

questions. All studies were scored to be at risk of bias. This was mostly due to uncertainty in the flow and timing domain. As concerned to flow, most studies did not explain withdrawals. As concerned to timing, time interval between reference standard and index test for diagnosis was not reported in most studies. In all studies the ANCA-testing and confirmation of the vasculitis was done using the same guidelines, so the differences in time interval are not of great influence. Therefore, we have not excluded studies based on the quality assessment, because we think that the risk of bias would not significantly influence the results.

There were a few studies that significantly stood out of the other studies with lower sensitivities and/or specificities in c-ANCA or anti-PR3. Hagen et al [13] had a low sensitivity compared to the other studies. This could be explained by a relatively large group of MPA patients, who have mostly a positive p-ANCA/anti-MPO instead of a positive c-ANCA/anti-PR3. Stone et al. [17] showed a relatively low sensitivity on both IIF and immunoassay. The authors explained this based on their study population. In their test population, a high prevalence of multiorgan system diseases was present that may easily mimic AAV. Combining IIF and immunoassay, however, did dramatically reduce their number of false-positive ANCA results.

In summary, based on the findings of this meta-analysis, we support the 2017 international European consensus that primary screening by anti-PR3 and anti-MPO immunoassay, without the need for ANCA testing by IIF, should be included in the workup towards a diagnosis of an ANCA-associated vasculitis [23]. Direct, capture and anchor immunoassay showed a comparable sensitivity and specificity. An anti-PR3 positive result would especially be of great value when GPA is suspected and an anti-MPO could be used for diagnosing MPA. In routine clinical practice, ANCA testing is not of great value as the prevalence of AAV is low and positive ANCAs also occur in more common diseases, like inflammatory bowel disease or rheumatoid arthritis. Therefore, suspicion of an AAV based on the clinical features is crucial for a correct interpretation of the ANCA serology. Requesting ANCA testing based on clinical features could also lead to a significant decrease of costs, as described by a recent French cost-efficiency evaluation study [50]. A biopsy remains the most reliable way to diagnose AAV. However, as that is not always possible and considering that a biopsy is invasive for the patient, ANCA serology takes an important place in the workup to the diagnosis of ANCA-associated vasculitis.

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Declaration of interest

None.

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None.

Appendix A

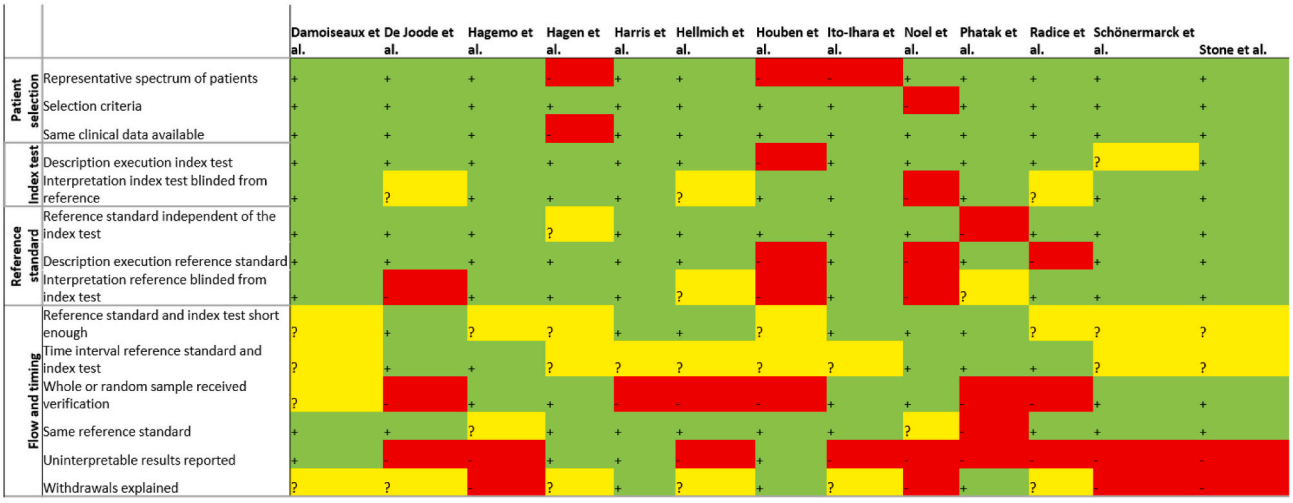

Fig. A.1. QUADAS-2 quality assessment.

Appendix B

Forest plot and SROC curve for anti-PR3 capture immunoassay.

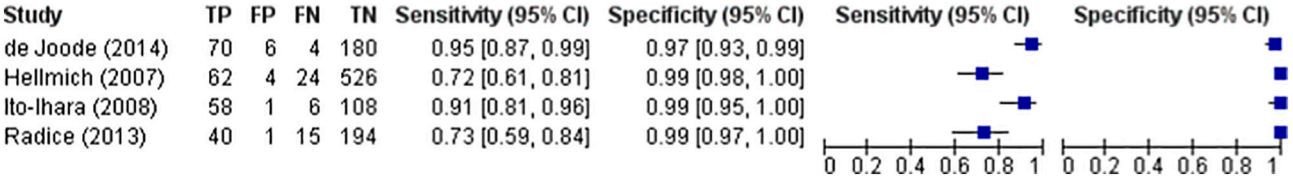

Fig. B.1. Forest plot: sensitivity and specificity for anti-PR3 capture immunoassay. TP (True Positives), FP (False Positives), FN (False Negatives) and TN (True Negatives) were calculated using the reported sensitivity, specificity, total subjects and positive tests.

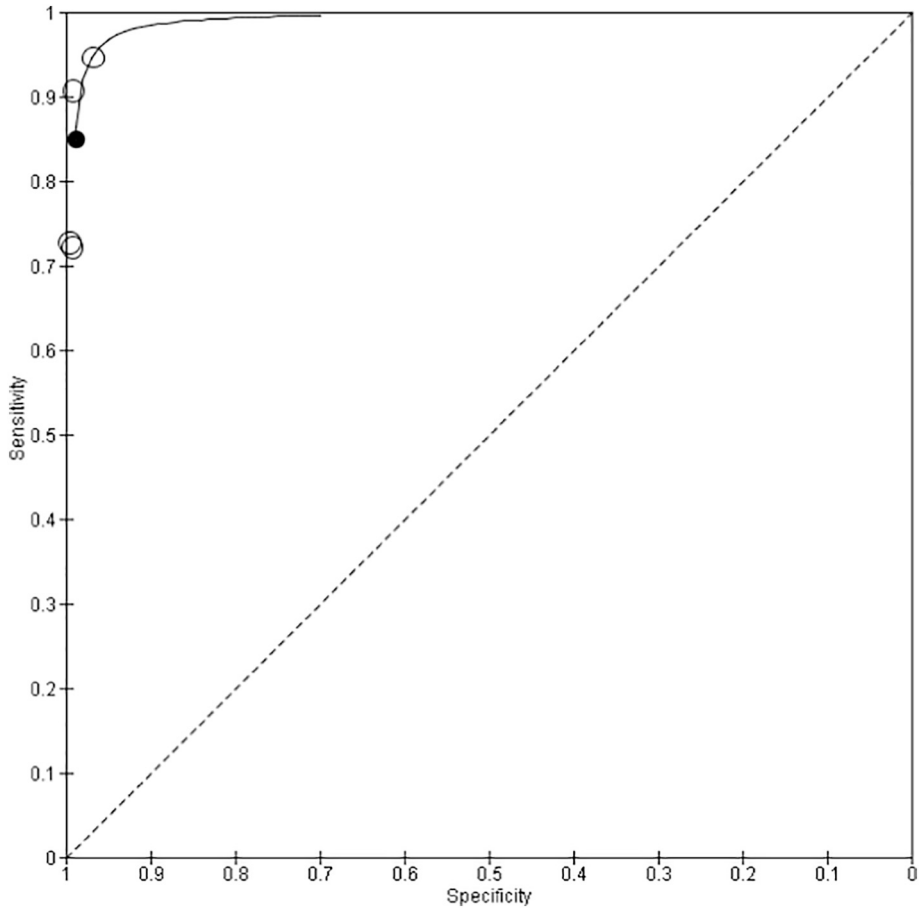

Fig. B.2. SROC curve for anti-PR3 capture immunoassay with pooled sensitivity (84.9%) and specificity (98.8%) with 95% CI.

Appendix C

Forest plot and SROC curve for anti-PR3 anchor immunoassay.

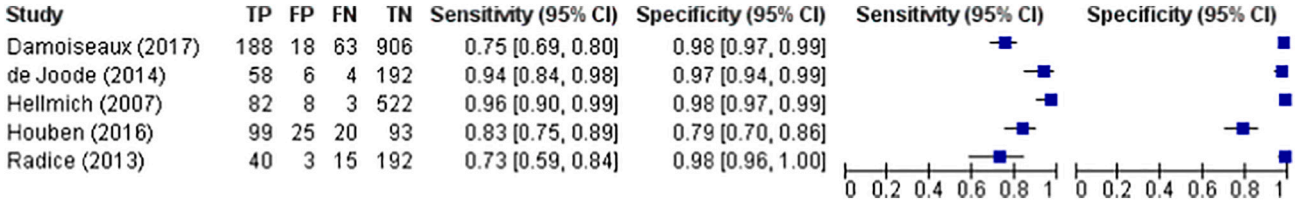

Fig. C.1. Forest plot: sensitivity and specificity for anti-PR3 anchor immunoassay. TP (True Positives), FP (False Positives), FN (False Negatives) and TN (True Negatives) were calculated using the reported sensitivity, specificity, total subjects and positive tests.

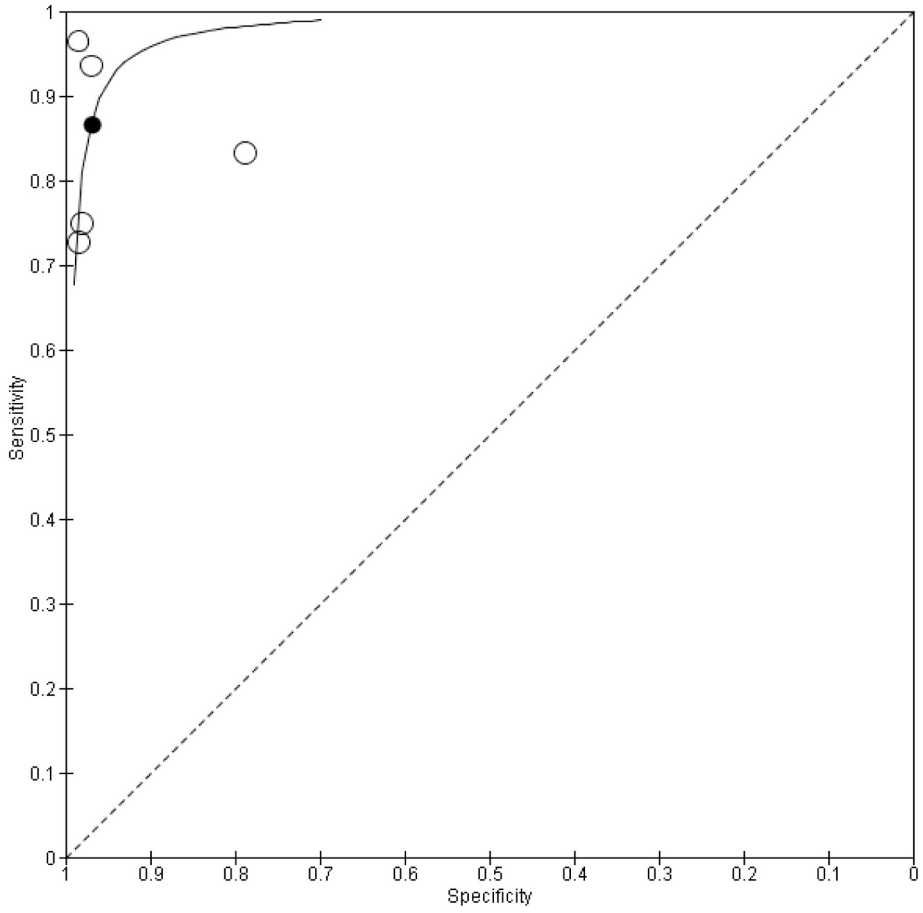

Fig. C.2. SROC curve for anti-PR3 anchor immunoassay with pooled sensitivity (86.6%) and specificity (96.8%) with 95% CI.

Appendix D

Forest plot and SROC curve for anti-MPO immunoassay.

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Damoiseaux (2017)	193	23	58	901	0.77 [0.71, 0.82]	0.98 [0.96, 0.98]
Hagemo (2002)	2	23	25	269	0.07 [0.01, 0.24]	0.92 [0.88, 0.95]
Hagen (1998)	100	83	175	841	0.36 [0.31, 0.42]	0.91 [0.89, 0.93]
Ito-Ihara (2008)	55	1	9	108	0.86 [0.75, 0.93]	0.99 [0.95, 1.00]
Schonermarck (2001)	182	30	202	4206	0.47 [0.42, 0.53]	0.99 [0.99, 1.00]

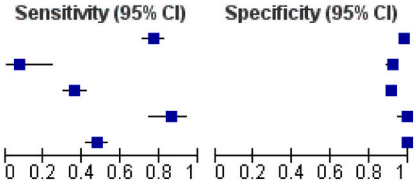

Fig. D.1. Forest plot: sensitivity and specificity for anti-MPO immunoassay. TP (True Positives), FP (False Positives), FN (False Negatives) and TN (True Negatives) were calculated using the reported sensitivity, specificity, total subjects and positive tests.

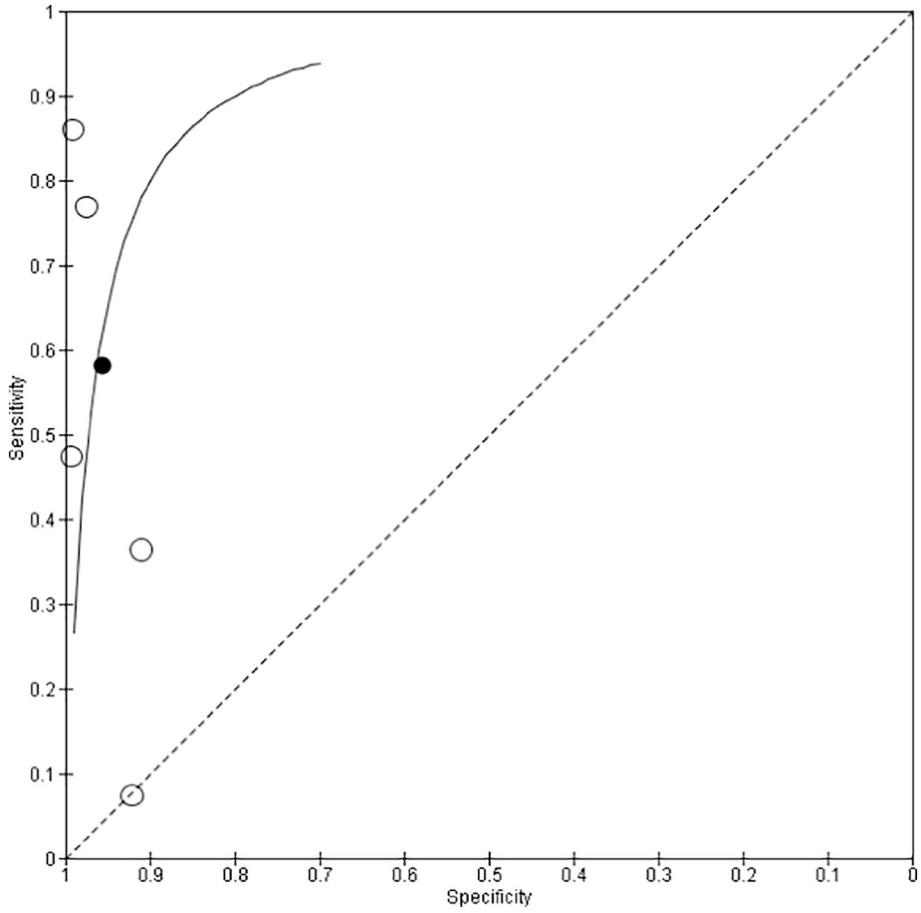

Fig. D.2. SROC curve for anti-MPO immunoassay with pooled sensitivity (58.1%) and specificity (95.6%) with 95% CI.

Appendix E

Eq. (1) Formula weighted average.

$$x = \frac{\sum_{i=1}^{n} w_i x_i}{\sum_{i=1}^{n} w_i} \tag{1}$$

With $i = \{$Granulomatosis with polyangiitis, Microscopic polyangiitis, Idiopathic RPGN, Classic polyarteritis nodosa, Eosinophilic granulomatosis with polyangiitis$\}$ and with w_i = numbers of patiënts with disease i and x_i = sensitivity of disease i.

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