Research article

Evaluation of Laboratory Test Ordering Practices for Patients Suspected of anti-Glomerular Basement Membrane Disease

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Background: **The objective of this study was to evaluate the laboratory test ordering practices for patients suspected of anti-glomerular basement membrane (anti-GBM) disease at an academic teaching hospital.**

Methods: **A retrospective cross-sectional study was conducted using data from EPIC electronic medical records (EMR) system from January of 2013 to January of 2022 on patients suspected of anti-GBM disease. Data collected include patient demographics, medical history, and laboratory test results. Patient data was stratified and analyzed using SPSS statistical software version 28.**

Results: **From the total 110 patients analyzed in this study; 42.7% (n=47) patients did not have an anti-GBM test ordered appropriately. Analysis of patient demographics revealed most of the patients were female (54.5%, (n=60)) and white (73.6%, (n=81)) non-Hispanic or Latino (69.1%, n=76)). Regarding type of anti-GBM serology tests, in the appropriate group, 41.3% (n=26 out of 63) of patients had both an enzyme-linked immunosorbent assay (ELISA) and indirect fluorescent antibody (IFA) test performed, while the inappropriate group 57.4% (n=27 out of 47) of patients had only an ELISA test ordered. There was a significant difference observed in serum creatinine (p= 0.003) and estimated glomerular filtration rate (eGFR) (p=0.011) for patients who had an anti-GBM test ordered appropriately.**

Conclusions: **The opportunities for quality improvement identified in this study can be used to implement a test ordering algorithm for anti-GBM to eliminate unnecessary diagnostic procedures and reduce hospital costs to improve patient outcomes.**

Keywords: Anti-glomerular basement membrane (anti-GBM), ANCA (anti-neutrophil cytoplasmic antibodies), autoimmune, serology, laboratory testing

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Introduction

Self-tolerance, which is the ability to differentiate self from non-self, is one of the most important characteristics of the immune system. Loss of self-tolerance can lead to autoimmune diseases which are characterized by the production of autoantibodies that bind to self-molecules leading to antigen-antibody deposits, cellular destruction, and tissue damage.[1] Anti-glomerular basement membrane (anti-GBM) disease, also referred to as Goodpasture syndrome, is an organ-specific autoimmune disorder marked by the production of autoantibodies against the glomerular and/or the alveolar basement membrane.[2] Specifically, the autoantibodies recognize and bind to the alpha-3 chain of type IV collagen, which activates the complement cascade and leads to tissue destruction.[3] Although basement membranes are found throughout the body, the basement membranes of the kidneys and lungs are predominately affected due to the structure of the alpha-3 collagen chains in the membranes are more exposed to antibodies.[2] Anti-GBM antibodies are not always associated with disease and can be present in healthy individuals. However, in patients with anti-GBM disease, the antibodies are potent and target two epitopes of type IV collagen leading to tissue destruction.[4]

Disease Incidence

Anti-GBM disease is considered rare with an incidence of 1-2 cases per million individuals.[4,5] A genetic predisposition for this disease is associated with human leukocyte antigen (HLA) allele, *HLA-DR15*, which is a common finding in other autoimmune diseases. Most patients with anti-GBM disease present with signs of progressive glomerulonephritis, in which most of the glomeruli have crescentic lesions.[6] Roughly, 40% to 60% of patients will also present with lung hemorrhage, and a small percentage will present with an isolated case of pulmonary disease.[7] Progressive glomerulonephritis in anti-GBM patients consists of renal damage, proteinuria, and glomerular hematuria. Lung hemorrhage or pulmonary disease presents as dyspnea or hemoptysis.[8]

Diagnosis of Anti-GBM Disease

Anti-GBM disease is primarily diagnosed by the detection of anti-GBM antibodies in serum or tissue.[7] Kidney biopsy is needed to confirm the diagnosis. However, biopsy is an invasive procedure and may not be possible in patients with severe cases of anti-GBM disease.[3] Guidelines published by Rovin et al, for the management of glomerular diseases conclude that treatment can start before biopsy, but biopsy confirms diagnosis.[9] In cases where kidney biopsy is not feasible, serum detection of anti-GBM antibodies is used. However, serological tests can produce false results and should only be ordered in patients with clinical suspicion of autoimmune disease.[10] Furthermore, due to difficulties in test result interpretation and insufficient knowledge among healthcare professionals regarding proper use of serology laboratory tests, autoantibody tests are often ordered unnecessarily.[11]

The prognosis of patients diagnosed with anti-GBM disease has improved over the last few years. However, most patients have limited renal survival and are dialysis dependent.[12] Thus, early diagnosis and treatment are essential for patients suspected of disease to prevent renal failure and death in severe cases.

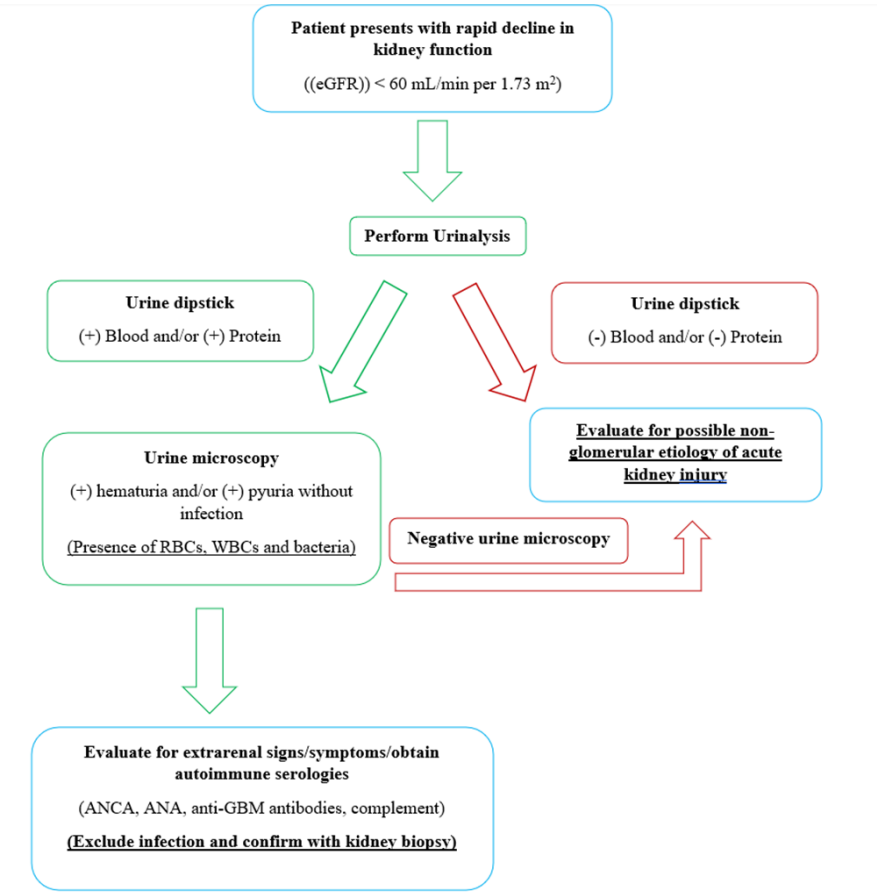
Purpose of Study

The lack of studies evaluating the efficient use of laboratory tests for patients suspected of anti-GBM disease led to the hypothesis that patients suspected of anti-GBM disease undergo unnecessary laboratory testing during the preliminary diagnostic process. In this study, individuals suspected of anti-GBM disease were defined as patients who presented clinically with glomerulonephritis and/or pulmonary hemorrhage. Laboratory test results investigated include serum albumin, creatinine, eGFR (estimated glomerular filtration rate), hemoglobin, hema-

tocrit, anti-GBM titer, antineutrophil cytoplasmic antibodies (ANCA), and urinalysis (urine dipstick and urine microscopy). The predictors of anti-GBM disease were identified using patient demographics, medical history, and laboratory test results. The objective of this project was to evaluate the current practices of test ordering for patients suspected of anti-GBM disease. The results from the study can aid in decreasing irrelevant testing, thus reducing costs for both the patient and the hospital.

Methods

This investigation consisted of a retrospective cross-sectional study using patients' EMR from January 2013 to January 2022 at an 800-bed academic teaching hospital. The study population included patients who clinically presented with glomerulonephritis and/or pulmonary hemorrhage suspected of having anti-GBM disease. Glomerulonephritis was defined by the International Classification of Diseases (ICD)10 and ICD9 codes, N00, N01, N02, N03, N04, N05, N06, N07, N08, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589. Pulmonary hemorrhage was defined by the ICD10 and ICD9 codes, R04, 786.3, 786.0. Anti-GBM disease was defined by ICD10 and ICD9 codes, M31.0 and 446.21. Analysis of the type of anti-GBM serology tests ordered included ELISA and IFA. Test results were interpreted as positive, negative, or indeterminate by the established reference standard of the specific test used. Patients less than 18 years of age, pregnant individuals, and prisoners were excluded from the study. The study was reviewed by the Institutional Review Board (IRB) and considered to be a quality assessment/quality improvement study that did not require approval or oversight.



Note. eGFR = Estimated glomerular filtration rate; RBC = Red blood cells; WBC = White blood cells; ANCA = Antineutrophil cytoplasmic antibodies; ANA = Antinuclear antibody

Figure 1. Determination of Appropriate Utilization of Laboratory Tests. Algorithm adapted from Rovin BH, Adler SG, Barratt J, et al. Executive summary of the KDIGO 2021 Guideline for the Management of Glomerular Diseases. Kidney Int. Oct 2021;100(4):753-779. doi:10.1016/j.kint.2021.05.015

During the chart review process, data collected included patient demographics (age, sex, ethnicity, race), patient medical history for pre-existing comorbidities (diabetes, dyslipidemia, smoking, hypertension), laboratory test results (urinalysis, creatinine, hematocrit, hemoglobin, eGFR) and type of anti-GBM test ordered (ELISA, IFA, or both). Assessment of appropriate laboratory test utilization in the anti-GBM disease diagnosis was accomplished by evaluating patient charts using the algorithm outlined in Figure 1.[9] Patients included in the study were divided into two groups, appropriate or inappropriate, based on the results of the algorithm evaluation.

Statistical Analysis

The statistical analyses were performed using IBM SPSS software version 28. Descriptive statistics were used to provide an overview of the patient population. Frequencies were determined for categorical variables including race/ethnicity and gender. Mean, median, and standard deviation were determined for continuous variables including age and laboratory test results. A Mann-Whitney *U* test was performed to compare laboratory test results between patients in the appropriate group and inappropriate group. *P* values < 0.05 were considered statistically significant.

Results

Between January 2013 and January 2022, 110 patients were evaluated for anti-GBM disease. Patients assessed for anti-GBM disease were primarily female (54.5%, (n=60)), white (73.6%, (n=81)), non-Hispanic or Latino (69.1%, n=76)) with a mean age of 52 years as seen in Table 1. Regarding frequency of comorbidities, hypertension (50.9%, (n=56)) was the most common condition, followed by smoking (37.3%, (n=41)) as listed in Table 2.

Following Rovin et al. algorithm, of the 110 patients suspected of anti-GBM disease, 63 (57.3%) patients had an anti-GBM test ordered appropriately, while 47 (42.7%) patients had tests ordered inappropriately as seen in Table 3.

Table 1. Frequency of sex, ethnicity, and race for patients suspected of anti-GBM disease.

Demographic	N (%)[n=110]
Age	
≥ 40 years	78 (70.9)
≤40 years	32 (29.1)
Sex	
Male	50 (45.5)
Female	60 (54.5)
Ethnicity	
Not Hispanic or Latino	76 (69.1)
Hispanic or Latino	33 (30)
Unknown	1 (0.9)
Race	
White	81 (73.6)
Black or African American	24 (21.8)
Asian	4 (3.6)
American Indian or Alaskan Native	1 (0.9)

Table 2. Frequency of comorbidities for patients suspected of anti-GBM disease.

Condition	N (%)[n=110]
Diabetes	
Present	23 (20.9)
Absent	87 (79.1)
Dyslipidemia	
Present	9 (8.2)
Absent	101 (91.8)
Smoking	
Present	41 (37.3)
Absent	69 (62.7)
Hypertension	
Present	56 (50.9)
Absent	54 (49.1)

Table 3. Frequency of appropriate and inappropriate ordering for anti-GBM tests.

Appropriate	N (%)[n=110]
Yes	63 (57.3)
No, No UA done	9 (8.2)
No, UA neg for protein	11 (10)
No, UA neg for blood	12 (10.9)
No UA neg for protein and blood	15 (13.6)

Note. UA = urinalysis; neg = negative

When assessing the frequency of the type of anti-GBM test ordered, the study identified, in the appropriate group, 26 of 63 (41.3%), had both an ELISA and an IFA test ordered. In the inappropriate group, 27 of 47 (57.4%), had only an ELISA test ordered as listed in Table 4.

Table 4. Type of anti-GBM serology test ordered.

Serology Test Ordered	Group	
	Appropriate Group *(n=63)*	Inappropriate Group *(n=47)*
Only ELISA	23	27
Only IFA	14	11
Both ELISA and IFA	26	9

Note. ELISA = Enzyme-linked immunosorbent assay; IFA = Indirect fluorescent antibody

When comparing laboratory tests between appropriate and inappropriate patient groups, the study found a statistically significant difference for serum creatinine (p = 0.003) and eGFR rate (p = 0.011). No statistically significant difference was seen for hematocrit (p = 0.059), hemoglobin (p = 0.67) and albumin (p = 0.131) as listed in Table 5.

Table 5. Comparison of laboratory tests between patients who had an anti-GBM test ordered appropriately and patients who had an anti-GBM test ordered inappropriately.

Variable	Mean for Appropriate Group	Mean for Inappropriate Group	p-Value
Creatinine (mg/dL)	4.41 *(n=66)*	2.81 *(n=37)*	0.003*
Hematocrit (%)	34 *(n=63)*	33.7 *(n=30)*	0.059
Albumin (g/dL)	3.47 *(n=62)*	3.69 *(n=29)*	0.131
eGFR (mL/min/1,73m^2)	3.34 *(n=57)*	58.7 *(n=34)*	0.011*
Hemoglobin (g/dL)	10.1 *(n=63)*	11.1 *(n=30)*	0.67

Note. eGFR = Estimated glomerular filtration rate

Discussion

The findings of this study indicate that 42.7% (n = 47) of patients suspected of having anti-GBM disease have inappropriate laboratory tests ordered according to the guidelines published by Rovin and colleagues.[9] The analysis of laboratory tests ordered (Table 3) showed that providers order anti-GBM serology tests without fully utilizing the results from patient's urinalysis. Unnecessary testing can be harmful to patients since it leads to diagnostic errors associated with inappropriate test results.[10,13] In patients suspected of anti-GMB disease, serology tests should only be ordered after assessment of clinical symptoms, comorbidities, and preliminary laboratory tests such as albumin, creatinine, eGFR, hemoglobin, hematocrit, and urinalysis.

Although, the patients included in the study were not diagnosed with anti-GBM disease, the demographics of the patients were found to be similar to other documented studies in which most patients were female, white not Hispanic or Latino.[7,14] Most patients in this study were female, but the study by Shen and others observed a male predominance in anti-GBM patients.[15]

In this study, the dominant comorbidity seen in patients was hypertension followed by smoking. This is in agreement with several other studies in which 34% of anti-GBM patients had hypertension or a history of hypertension and 58.3% of anti-GBM patients had hypertension or a history of hypertension.[12,14] Hypertension is the most common comorbidity that is associated with anti-GBM disease. This can be the result of early glomerular lesions associated with fibrin deposition and formation of epithelial crescents that narrow the blood vessels leading to kidney damage presenting initially as hypertension. Also, environmental factors, mainly smoking, increases the risk of developing anti-GBM disease as smoke damage to the pulmonary membranes leads to exposure of the alveolar capillaries to anti-GBM antibodies.[2] It is important to note that anti-GBM tests performed in this study's population (n=110) were all negative and anti-GBM disease was not the final diagnosis. However, when considering initial evaluation of a patient suspected of anti-GBM, patient demographics and comorbidities identified are variables that should be considered. For example, a multicenter French study that had a total of 201 patients diagnosed with anti-GBM disease identified 57% of the study population was male and the two common comorbidities were chronic arterial hypertension and tobacco use.[16] Patient demographics and more importantly, existing comorbidities, can be informative to healthcare professionals in the initial evaluation of patients suspected of anti-GBM disease.

In terms of laboratory testing, ELISA and IFA were the two types of anti-GBM tests utilized and these tests were ordered either as a panel or individually. The anti-GBM tests were performed by a reference laboratory, it is possible that ordering providers overlooked Rovin et al testing algorithm in order to attempt faster turnaround time on test results.[9] This practice could have contributed to the inappropriate test ordering for the 47 patients in this study. Additionally, because the tests are offered as a panel, ordering providers could be prompted to order the panel rather than individual tests, especially if the provider is unfamiliar with the listed test.

Furthermore, when the test order recommendations listed in the reference laboratory website were reviewed, the guidelines for ordering a type of anti-GBM serology test (ELISA, IFA, or both) was unclear. For example, for the anti-GBM IFA tests, the reference laboratory recommends that this test may be useful in detecting GBM antibodies. However, the anti-GBM ELISA and IFA combo is listed as the preferred panel for detecting GBM antibodies in suspected or established anti-GBM disease. Interestingly, the result interpretations provided by the reference laboratory regarding anti-GBM IFA or ELISA and combo, are identical. This confusion could be a reason providers decide to order both tests. There was not a clear ordering pattern for disease diagnosis as indicated by the variation in ELISA and IFA test ordering for patients in both the appropriate group and the inappropriate as seen in Table 4. Errors in test ordering could be related to the ambiguous information presented in the reference laboratory website, especially if the result interpretation provided is similar for all anti-GBM test types.

The comparison of laboratory tests completed between patients in the appropriate group and patients in the inappropriate group demonstrated a significant difference for creatinine and eGFR. The average creatinine for patients in the appropriate group was 4.41 mg/dL, while the average creatinine for patients in the inappropriate group was 2.81 mg/dL (Table 6). The average eGFR for patients in the appropriate group was 34.3 mL/min/1.73m^2, while the average eGFR for patients in the inappropriate group was 58.7 mL/min/1.73m^2 (Table 6). The average results for creatinine and eGFR for patients in the appropriate group and patients in the inappropriate group were abnormal. However, patients in the appropriate group had drastic abnormal results than patients in the inappropriate group. This is significant, as anti-GBM disease is associated with severe kidney injury as many patients with delayed diagnosis require permanent renal replacement therapy.[17]

The findings from the study indicate that patients who had an anti-GBM test ordered appropriately were experiencing more severe kidney disease as observed by the mean eGFR which would place them in moderate (3b) to severe chronic kidney disease (CKD) stage. Patients who had an anti-GBM test ordered inappropriately had an eGFR mean that would indicate mild to moderate (3a) CKD stage. These results may be useful in establishing cut off values for anti-GBM test ordering, as patients who had more abnormal values for creatinine and eGFR had an anti-GBM test ordered appropriately.

The contribution of this study notes a high volume of tests ordered inappropriately for patients suspected of anti-GBM disease despite a low prevalence of disease. Leaf et al, evaluated 4,903 patients with 5,731 acute kidney injury (AKI) episodes and identified that anti-GBM antibodies were tested in 1% of AKI episodes and all were found to be negative.[18] Since the prevalence for anti-GBM disease is low, greater emphasis should be placed on patient signs and symptoms, comorbidities, and routine laboratory tests such as urinalysis, creatinine and eGFR before performing autoantibody testing.

While the preferred testing for initial diagnosis in patients suspected of anti-GMB disease include GBM antibody testing by IgG by multiplex bead assay and immunofluore-

scence, it should be noted that other laboratory tests along with renal biopsy must be included for proper diagnosis. This is of particular interest as extremely rare cases of anti-GBM can present with seronegative anti-GBM antibodies.[19] Hospitals should consider newly developed methodologies, such as anti-GBM IgG chemiluminescence immunoassay as part of the anti-GBM workflow. When implemented appropriately, these assays have shown increased detection of GBM antibodies in addition to traditional ELISA testing.[20] Additionally, novel interventions could include educational seminars, built in test ordering sets within EMR, and dissemination of educational pamphlets detailing anti-GBM laboratory workup along with test costs to various departments of a hospital. Lastly, as part of on-going quality improvement, institutions should evaluate implemented interventions with a follow-up period of 3-6 months to assess the effectiveness of an intervention and modify as needed to continually improve patient outcomes while reducing hospital costs.

Conclusion

There is no evident diagnostic algorithm for anti-GBM that is available to providers. This may have contributed to improper test ordering. The results from this study should encourage institutions to evaluate the current practices in the diagnosis of anti-GBM testing and implement evidence-based diagnostic algorithms that can aid providers with laboratory test ordering for improved patient outcomes.

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Ethical Approval

This study was reviewed the IRB and considered it to be a quality assessment/quality improvement study that did not require IRB approval or oversight.

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