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Clinical characteristics of patients with diffuse alveolar hemorrhage diagnosed by cytological examination of 1000 bronchoalveolar lavage samples

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Abstract. *Background and aim:* Diffuse alveolar hemorrhage (DAH) is a life-threatening condition due to the extravasation of blood in the alveoli, resulting in hypoxemia and even acute respiratory distress syndrome. This study aimed to describe the clinico-radio-pathological profile of patients diagnosed with DAH and classify it into immune and nonimmune DAH. *Methods:* This was a retrospective analytical study. Of a total of 1000 cases of bronchoalveolar lavage fluids (BALF) received for cytological examination, patients fulfilling the clinical, radiological, and laboratory details of cases satisfying the clinical and cytological criteria of DAH (n=47) were studied. *Results:* The most common cause of immune DAH was ANCA-associated vasculitis (n=13, 27.6%), and that of nonimmune DAH was infections (n=10, 21.3%). Twenty-nine patients (61.7%) had hemoptysis. The most common radiological finding was ground-glass opacities (n=33, 70.2%). In univariate analysis, female sex, mean hemoglobin at admission, total leucocyte count (TLC), platelet count, and erythrocyte sedimentation rate (ESR) were significantly associated with immune-DAH. However, in multivariate analysis, female sex, higher TLC, high platelets, and high ESR were significantly associated with immune DAH. Patients were treated with corticosteroids (n=25, 46.3%), intravenous cyclophosphamide (n=12, 22.2%), plasma exchange (n=7, 13.0%), intravenous immunoglobulin (n=5, 9.3%) and rituximab (n=5, 9.3%). The overall mortality was 8.5% (n=4). *Conclusions*: DAH is a life-threatening syndrome that may be classified into immune and nonimmune DAH. Immune-DAH requires aggressive management, whereas nonimmune DAH cases respond best to conservative management.

Key words: Pulmonary hemorrhage, Alveolar hemorrhage, Idiopathic pulmonary hemosiderosis, Anti-Glomerular Basement Membrane Disease, Autoimmune Diseases, Antineutrophil Cytoplasmic Antibodies, Vasculitis

Introduction

Diffuse alveolar hemorrhage (DAH) is a life-threatening condition caused by the extravasation of blood into alveolar spaces. Accumulation of blood in the alveoli causes a diffusion barrier, resulting in hypoxemia, often progressing to acute respiratory distress syndrome (ARDS)(1). Repeated episodes of DAH may result in interstitial fibrosis, decreasing static and dynamic lung compliance (2,3). The symptoms of DAH include dyspnea, hemoptysis, cough, and fever. The severity of symptoms may range from mild dyspnea to severe hemoptysis (4). The diagnosis of DAH is based on clinical features, especially bilateral pulmonary infiltrates with or without a decrease in hemoglobin. Definitive diagnosis is established by bronchoscopic lavage physically by a bloody return on bronchoaleolar lavage (BAL). Alveolar macrophages phagocytose extravasated red blood cells and convert them to hemosiderin, which accumulates in macrophages and can persist in the lungs for up to 8 weeks (5). The histopathology of DAH is

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characterized by intra-alveolar RBCs and fibrin, with the accumulation of hemosiderin-laden macrophages (HLM) or siderophages (6-9). Localized alveolar hemorrhage (LAH) is caused by neoplasms, infections, necrotizing pneumonia, tuberculosis or fungal infections, anticoagulant medication, or bronchiectasis, whereas DAH is caused by diverse systemic disease states. The presence of HLM after an episode of silent bleeding confirms the diagnosis of DAH (7).

Many systemic diseases can cause DAH, but the pathogenesis is not well understood in the majority of cases. The presence of hemorrhagic bronchoalveolar lavage, HLM, or an increase in carbon monoxide diffusing capacity has been described in some series as helpful findings for the diagnosis.

Bronchoalveolar lavage fluid (BALF) is now the main diagnostic method for the identification of alveolar hemorrhage (AH) in routine clinical practice. The presence of recent hemorrhage (increased number of siderophages) is indicative of DAH. The most commonly employed criterion is the presence of ≥20% HLM in BALF, which correlates well with the Golde score (9,10). Lassence et al defined AH as at least 20% siderophages (7).

Few studies have been performed thus far in relation to DAH, available mostly as isolated case reports. In the present study, which to the best our knowledge, is the largest cohort of DAH described to date, we aimed to study the clinicoradiopathological profile of patients presenting with diffuse alveolar hemorrhage and compare immune DAH with non-immune DAH. As there is no consensus diagnostic scheme for the classification of DAH, we have tried to classify the disease.

Material and methods

Study design and ethics

This was a single-centre retrospective analytical study conducted from October 2015 to March 2021 at SGPGIMS, Lucknow, Uttar Pradesh, India. The STROBE guidelines for observational studies were used for reporting. The study was approved by the Institutional Ethics Committee (IEC).

(IEC Reference Code #2022-19-IMP-EXP-45).

Inclusion and exclusion criteria

All cases from which the BALF was sent to the cytopathology department from the pulmonary medicine ward were retrieved. One thousand BALF samples were evaluated, and their cytology smears were reviewed. Patients fulfilling both clinical and cytological diagnoses of DAH were included in the analysis, while patients with inadequate cytological samples or inadequate records were excluded from the study. The selection of study cases is depicted in Figure 1.

Cytological examination

The criteria for the inadequacy of BALF that were adopted were as described by Chamberlain et al (11). So, the following BAL specimens were excluded from study 1) paucity of alveolar macrophages on prepared glass slides (less than 10 alveolar macrophages per high-power fields or less than 25 alveolar macrophages per high-power field in combination with either criterion two or three); 2) an excessive number of epithelial cells, 3) a mucopurulent exudate; 4) numerous red blood or haemorrhagic BAL or 5) artefacts.

Twelve specimens were inadequate and excluded from the study. Cases with adequate alveolar macrophages were incorporated into the study and included those with a clinical-radiological diagnosis of DAH (n=47). The clinical and laboratory data were obtained from the hospital information service system and patient record files. HLM was detected in BALF by cytological examination. The patients were classified into two groups, immune and non-immune DAH, based on underlying etiology. The immune group included cases where an underlying autoimmune disease was established as the aetiology based on serological and clinical profiles. The nonimmune group included cases secondary to infections, pulmonary artery hypertension (PAH), idiopathic pulmonary hemosiderosis and miscellaneous with no identifiable cause.

Statistical analysis

STATA 17.0 (StataCorp. 2021. *Stata Statistical Software: Release 17*. College Station, TX: StataCorp LLC) and GraphPad Prism (GraphPad Prism version 9.4.0 for Windows, GraphPad Software, San Diego, California USA) were used for the data analysis. Most of the data were not normally distributed. Continuous variables are presented as the mean and standard deviation for normally distributed data and the median and interquartile range (IQR)

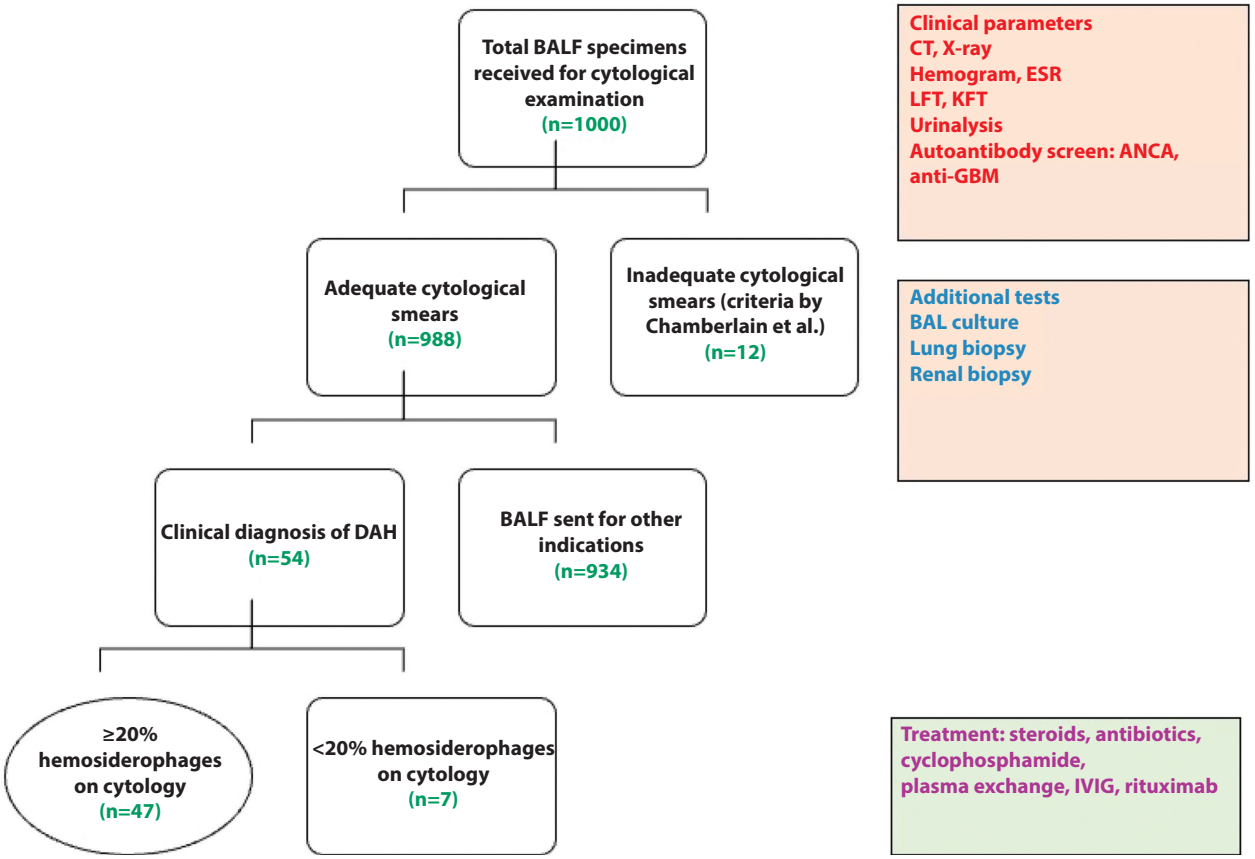

Figure 1. Selection of study cases. BALF: bronchoalveolar lavage fluid, AH: alveolar hemorrhage, CT: computed tomography, ESR: erythrocyte sedimentation rate, LFT: liver function tests, KFT: kidney function tests, ANCA: anti-neutrophil cytoplasmic antibodies, anti-GBM: anti-glomerular basement membrane, IVIG: intravenous immunoglobulins.

for nonnormal data. Categorical variables were described as numbers and percentages. The Mann–Whitney U test was used to compare continuous variables. Fisher's exact test was used to compare categorical variables. A two-sided p value of less than 0.05 was considered significant. Multivariate analysis by binary logistic regression with a backwards-wald model was used to identify significant variables. All the variables that were found to be significant in the univariate analysis were included in the model.

Results

The distribution of cases according to aetiology is summarized in Figure 2 and Table 1.

Patient demographics

The mean age of the patients was 39.4±17.2 years (range 13–73 years). Of these, 61.7% (n=29) were male. A total of 8.5% (n=4) had a smoking history, and 8.5% (n=4) were immunocompromised. The causes of the immunocompromised state were renal transplantation (n=1) and immunosuppressive therapy (n=3). Symptoms at presentation included cough in 44.7% (n=21), hemoptysis in 61.7% (n=29), dyspnea in 55.3% (n=26) and fever in 40.4% (n=19) of cases. Female sex was significantly correlated with immune DAH (p value 0.033).

Laboratory parameters

All patients had peripheral blood analysed for complete blood cell counts during the BAL procedure. There was a significant drop in hemoglobin from admission to diagnosis (1.5 (1.1, 2.6), p<0.05), but this drop was similar in both groups. The mean hemoglobin level in the immune DAH group was lower than that in the nonimmune DAH group (p value 0.018).

ANA was done by immunofluorescence method, 3 patients in the immune group had ANA

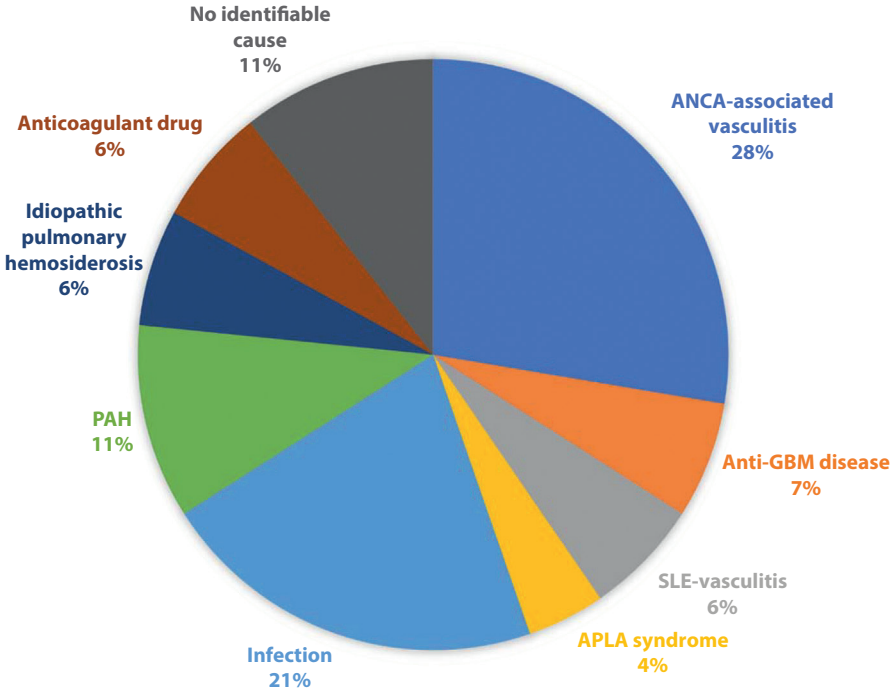

Figure 2. Causes of diffuse alveolar hemorrhage. (N=47) The most common cause was antinuclear antibody-associated vasculitis (13). Other causes were pulmonary infections (10), pulmonary arterial hypertension (5), idiopathic pulmonary hemosiderosis (3), anti-glomerular basement membrane-associated disease (3), systemic lupus erythematosus (3), anticoagulant drugs (3), antiphospholipid antibody syndrome (2), and no identifiable cause (5).

Table 1. Comparison of various parameters between the two groups.

	Total (N=47)	Immune DAH (N=21)	Non-Immune DAH (N=26)	Odds-Ratio (95% CI)	p-value
Age (years)	36 (25-54)	40(22.5-55)	35.5(24.5-52.75)	1.01 (0.97-1.04)	0.710
Females	18 (38.30)	12 (57.14)	6 (23.08)	4.44 (1.26-15.62)	0.033*
Cough	21 (44.68)	11 (52.38)	10 (38.46)	1.76 (0.55-5.64)	0.388
Fever	19 (40.43)	9 (42.86)	10 (38.46)	1.2 (0.37-3.87)	0.774
Hypertension	9 (19.15)	4 (19.05)	5 (19.23)	0.99 (0.23-4.26)	1.000
Diabetes mellitus	10 (21.28)	4 (19.05)	6 (23.08)	0.78 (0.19-3.25)	1.000
Smoker	4 (8.51)	1 (4.76)	3 (11.54)	0.38 (0.04-3.98)	0.617
Mortality	4(8.51)	3(14.28)	1(3.85)	4.17(0.44-43.38)	0.233
Investigations					
Hemoglobin at admission (g/dL)	9.6(8.6-12.5)	9(8.4-10.35)	11.85(9.18-12.8)	**0.69 (0.50-0.94)**	**0.018**
Hemoglobin at diagnosis (g/dL)	8(6.9-9.9)	7.7(6.85-8.45)	8.55(7.2-10.85)	0.73(0.53-1.01)	0.058
Hemoglobin drop (g/dL)	1.5(1.1-2.6)	1.5(1.1-2.5)	1.5(1.18-2.65)	0.84 (0.57-1.50)	0.553
TLC ($\times 10^3/\mu L$)	9.1(6.9-12.6)	9.1(6.8-15.2)	8.7(6.88-10.18)	**1.19 (1.01-1.40)**	**0.040***
Platelet count ($\times 10^3/\mu L$)	160(122-250)	250(156-337)	151(108-163)	**1.02 (1.01-1.03)**	**0.005***
ESR (mm/hr)	69(30-92)	88(69-108.5)	40(24.75-69)	**1.04 (1.02-1.07)**	**0.001***
ESR>40 mm/hr	34 (72.34)	19 (90.48)	15 (57.69)	**6.97 (1.34-36.34)**	**0.020**

	Total (N=47)	Immune DAH (N=21)	Non-Immune DAH (N=26)	Odds-Ratio (95% CI)	p-value
Creatinine >1.6 mg/dL	25 (53.19)	8 (38.10)	17 (65.38)	0.33 (0.99-1.08)	0.082
INR	1.23(1.12-1.33)	1.26(1.11-1.33)	1.23(1.12-1.33)	0.33 (0.01-76.98)	0.702
AST >40 (U/L)	29 (61.70)	12 (57.14)	17 (65.38)	0.71 (0.22-2.30)	0.763
ALT >40 (U/L)	33 (70.21)	17 (80.95)	16 (61.54)	2.66 (0.69-10.20)	0.205
Urine					
RBC>5/hpf	18 (38.30)	11 (52.38)	7 (26.92)	2.99 (0.88-11.00)	0.130
WBC>5/hpf	9(9.15)	4 (44.4)	5 (55.6)	1.22 (0.33-5.29)	0.999
Proteinuria (>100mg/dL)	19 (40.43)	11 (52.38)	8 (30.77)	2.48 (0.75-8.17)	0.151
Radiological					
Bilateral ground glass opacities	33 (70.21)	17 (80.95)	16 (61.54)	2.66 (0.69-10.20)	0.205
Multifocal consolidation	10 (21.28)	4 (19.05)	6 (23.08)	0.78 (0.19-3.25)	1.000
Pleural effusion	3 (6.38)	2 (9.52)	1 (3.85)	2.63 (0.22-31.22)	0.590
Cavitation	2 (4.26)	-	2 (7.69)	-	0.495
BAL					
Macrophages (%)	84(64-86)	84(82.5-86.5)	84(61-86.25)	1.04 (0.99-1.10)	0.146
Lymphocytes (%)	84 (63.75- 86.25)	9(9-10.5)	9(8.75-11.25)	1.05 (0.86-1.28)	0.647
Neutrophils (%)	1(1-2)	1(1-2)	1(1-4.25)	0.97(0.91-1.03)	0.305
Epithelial cells (%)	3(2-7)	4(3-6)	3(2-8)	0.97 (0.90- 1.04)	0.375
Siderophages (%)	61(43-83)	61(40.5-82.5)	63(41.5-93)	0.99 (0.97-1.01)	0.476
Histopathology					
Lung biopsy	6 (12.77)	-	6 (100)	-	-
Renal biopsy	14 (29.79)	8 (38.10)	6 (42.9)	10.27 (2.38-43.54)	0.007
Autoantibodies					
ANA	10(21.28)	3 (14.29) 4+ at 1:100	7 (26.92) 1+at 1:80 (4) 2+(4) at 1:100(3)	0.56 (0.14-2.34)	0.718
p-ANCA IIF: 4+ Anti-MPO >100	8(17.02)	8 (38.10)	-	-	-
c-ANCA IIF: 4+ Anti-PR-3 >100	5(10.64)	4 (19.05)	1 (3.85)	7.20 (0.95-90.71)	0.142
Anti-GBM antibodies	3(6.38)	3 (14.29)	-	-	-
APLA	2(4.26)	2(9.52)	-	-	

Data presented in median (Q1, Q3) [Mean+SD], compared by Mann–Whitney U test. Number (%), compared by Fisher exact test. **(P<0.05 significant)**

CECT: contrast-enhanced computed tomography, Hb: Hemoglobin, TLC: total leucocyte count, ESR: erythrocyte sedimentation rate, ALT: alanine transaminase, AST: aspartate aminotransferase, RBC: red blood cells, ANA: anti-nuclear antibodies, p-ANCA: perinuclear anti-neutrophil cytoplasmic antibodies, c-ANCA: cytoplasmic anti-neutrophil cytoplasmic antibodies, anti-GBM: anti-glomerular basement membrane.

*Significant by binary logistic regression: Sex: 15.74(1.55-159.35), p=0.020; ESR: 1.04(1.01-1.07), p=0.015; Platelet count 1.02(1.01-1.03), p=0.028.

4+ homogeneous pattern at 1:100 whereas, in the non-immune group four patients had 1+ speckled pattern at 1:80, while three patients had 2+ fine speckled pattern at 1:100. ANCA was also done by immunofluorescence method. Eight patients had 4+ on immunofluorescence with anti-myeloperoxidase >100 units/ml, five patients had 4+ on immunofluorescence with anti-PR-3 >100 units/ml

Chest radiography and computed tomography

High-resolution computed tomography (HRCT) of the chest were available for all patients and revealed bilateral ground-glass opacities in 70.2% (n=33) of the cases (Fig 3A) and multifocal consolidation in 23.4% (n=11). Uncommon findings were pleural effusion in 6.4% (n=3) (Fig 3B), cavitatory lesions in 2.1% (n=1) (Fig 3A), fibronodular opacities in 4.3% (n=2) and bronchiectasis in 2.1% (n=1).

Bronchoalveolar lavage fluid analysis

The mean proportion of HLMs in BALF was 56.4 ± 29.1% (range 0–96%). On differential cell count, alveolar macrophages were the predominant cell type, with a mean percentage of 77.8±12.1. Lymphocytes, neutrophils, and epithelial cells accounted for 8.9±3.0, 5.8±10.6, and 7.5±8.8%, respectively.

Etiologies

The causes of DAH (n= 47) were immune and nonimmune. The immune DAH group included antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis [AAV] in 27.6% (n=13): p-ANCA in 8, c-ANCA in 5 cases, anti-glomerular basement membrane [anti-GBM] disease in 6.4% (n=3), systemic lupus erythematosus [SLE] vasculitis in 6.4% (n=3) and antiphospholipid antibody syndrome [APLA] syndrome in 4.3% (n=2). The nonimmune DAH group included infections in 21.3 (10%), *pulmonary arterial hypertension* [PAH] in 4.3% (n=2), idiopathic

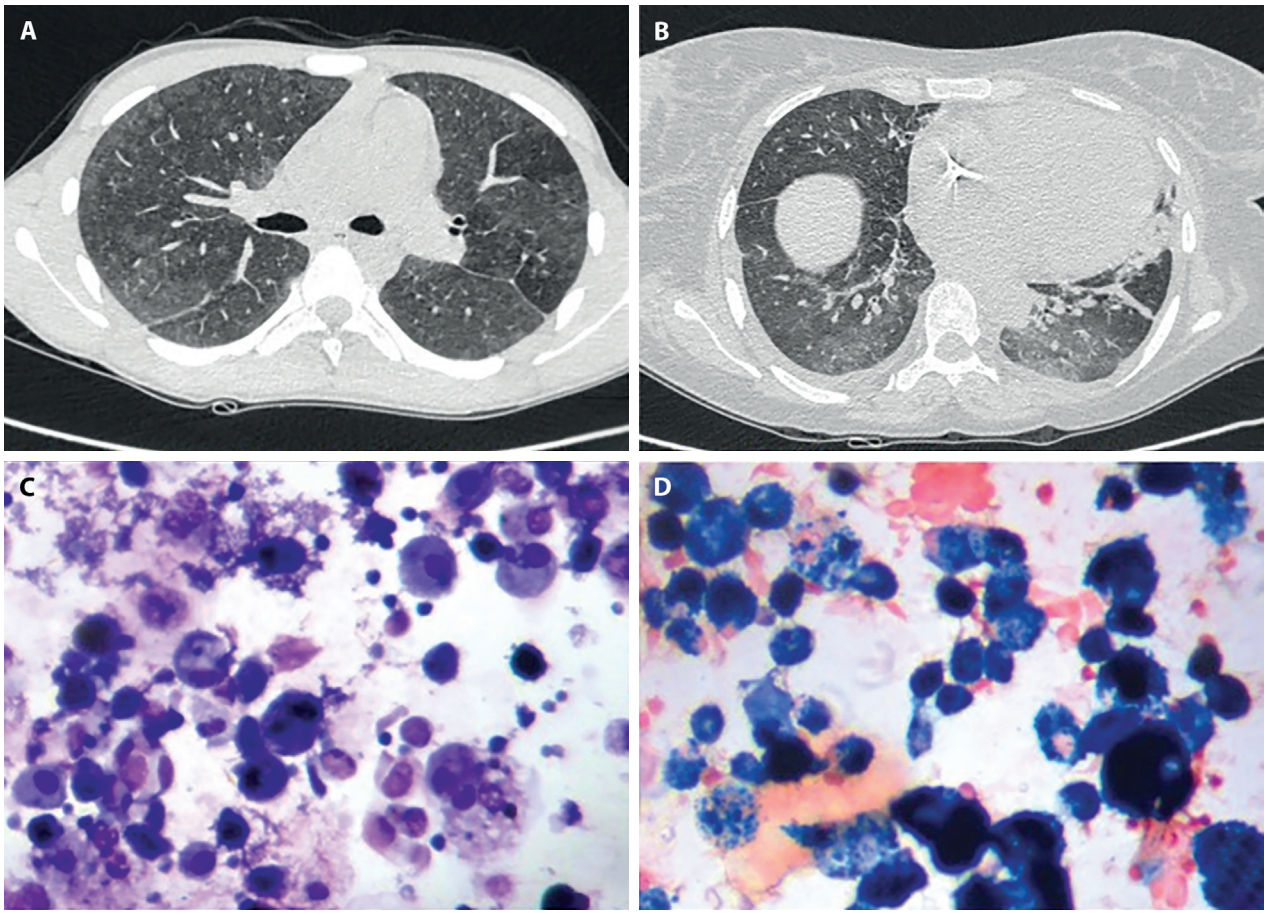

Figure 3. A: CT scan from a case of DAH displaying bilateral geographical ground-glass opacities. B: CT scan from another case displaying bilateral geographical ground-glass opacities along with right-sided pleural effusion. C: BAL fluid cytology smear from a case showing alveolar macrophages containing abundant blackish intracytoplasmic pigment (MGG x200). D: Cytology smear showing hemosiderophages containing cytoplasmic bluish-green pigment (Perls' stain x400).

pulmonary hemosiderosis in 6.4% (n=3) and anticoagulant therapy in 6.4% (n=3). A total of 10.6% (n=5) of cases were classified as miscellaneous and included those without a definite cause, despite extensive clinical and laboratory workup, which was not firmly established. (Fig 2)

Lung biopsy was available in six cases (12.7%), of which three were diagnosed as pulmonary hemosiderosis and one as organizing pneumonia, granulomatous lesion favoring vasculitis, and aspergillosis. Renal biopsy was available in fourteen (29.8%) cases, of which eleven were diagnosed as crescentic glomerulonephritis (nine ANCA-associated glomerulonephrites and two anti-GBM disease), one as advanced diabetic nephropathy even though the patient had AAV, one case had a membranoproliferative glomerulonephritis pattern, and one was a graft biopsy, which revealed chronic active antibody-mediated rejection with focal segmental glomerulosclerosis.

Treatment

Immunosuppressive therapy was given to 55.3% (n=26) of DAH cases, which included steroids (55.3%, n=26) with or without cyclophosphamide (22.2%, n=12%), rituximab (9.3%, n=5), intravenous immunoglobulins (9.3%, n=5), or plasmapheresis (13.0%, n=7). The remaining 44% (n=21) of DAH cases did not receive immunosuppressive therapy, as their disease severity was relatively low, and the adverse risk associated with immunotherapy outweighed the benefit.

Mortality

Overall mortality was 8.5% in the immune group and 14.28 vs 3.85% in the nonimmune group. However, this difference was not statistically significant.

Univariate and multivariate analysis

Female sex, lower hemoglobin at admission, higher TLC, higher platelets, a high ESR, were found to significantly associated with immune DAH. However, on multivariate analysis, female sex, a high ESR and a higher platelet count were found to be significant. (Figure 4)

Discussion

Diffuse alveolar hemorrhage (DAH) is a life-threatening condition characterized by hemoptysis, anemia, and diffuse alveolar infiltrates. The diagnosis of DAH is generally established by BAL along with radiology and serology in the appropriate

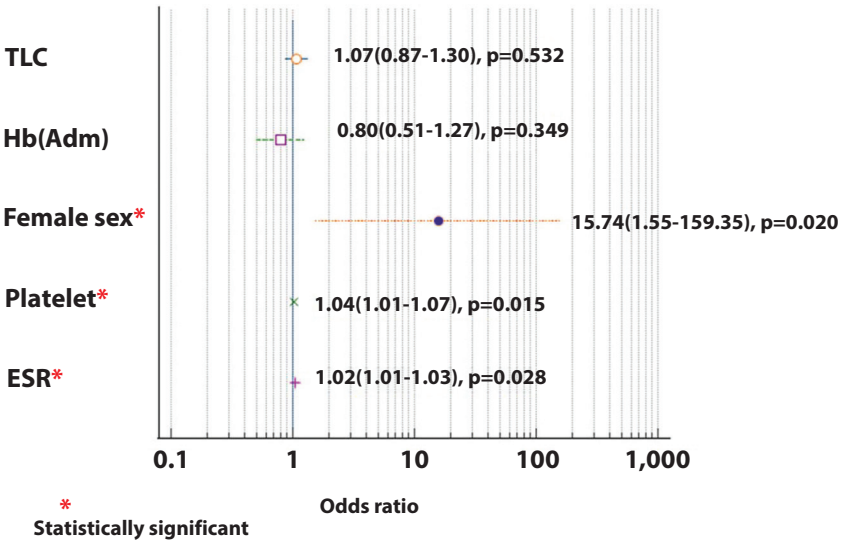

Figure 4. Multivariate analysis for factors leading to the diagnosis of immune DAH. Variables found to be significant in univariate analysis were included in the binary logistic regression model: female sex, a low hemoglobin, high platelet count, high total leukocyte count, high erythrocyte sedimentation rate (ESR) at admission. Out of these, female sex, a high ESR and a higher platelet count were found to be significantly associated with immune DAH on multivariate analysis.

clinical setting. Renal biopsy and lung biopsy may be required if vasculitis or anti-GBM disease is being considered to guide the therapy and prognosticate the disease. The majority of cases of DAH are caused by vasculitis, which may be primary idiopathic small vessel vasculitis, primary immune-complex mediated, or secondary. Coagulation disorders, drugs, inhaled toxins, or transplantation are other known causative factors. In our series, the most common cause of DAH was vasculitis. DAH has traditionally been linked to systemic vasculitides (12). Quadrelli et al. characterized 39 patients with DAH of proven immunological etiology, the most frequent being ANCA-related vasculitides (74%), mainly granulomatosis with polyangiitis (GPA) and microscopic polyangiitis (MPA). In their series, these two diseases accounted for a similar number of cases (14 GPA *vs.* 13 MPA). Alexandre et al. found that ANCA-related vasculitides were the most common cause of immune-related DAH (~73%) (13). AAV was the most common cause in our study, followed by anti-GBM disease, SLE vasculitis, and one case of unclassifiable vasculitis.

Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitides (AAV) are a group of systemic diseases marked by inflammation and necrosis of small and medium-sized arteries, and one of the most common symptoms is pulmonary involvement. The damage to the alveolar basement membrane resulting from the widespread injury of the pulmonary capillaries promotes the extravasation of red blood cells into the pulmonary alveolar spaces. DAH affects approximately 7–45% of GPA and 10–30% of MPA patients, but is uncommon in EGPA patients. The severity of the disease varies from life-threatening to milder types, with associated renal impairment occurring in up to 97% of cases (14). The frequency of DAH was 8–36%, and 57% of the patients showed anti-proteinase-3 (PR3) antibodies, according to a systematic analysis by West et al., which comprised nine studies with a total of 207 patients (15). ANCA antibodies were found to be positive in 13 cases in our study. DAH is the most common and the most severe form of pulmonary manifestation of anti-GBM disease. The molecular and nephritogenic properties of anti-GBM antibodies that bind to renal glomeruli and alveolar-capillary basement membranes are responsible for causing glomerulonephritis and DAH, respectively (16). The exact pathogenesis of DAH in SLE is not fully understood. Diffuse alveolar bleeding, interstitial inflammation, and alveolar hemorrhage without vasculitis are the histopathology findings mentioned. Other reports, on the other hand, have revealed pulmonary vasculitis, with evidence pointing to pulmonary capillaritis and microangiitis as the source of DAH in SLE patients (17).

The nonimmune causes include infections, APLA syndrome, PAH, idiopathic pulmonary hemosiderosis, and miscellaneous. The main infections that induce DAH in immunocompromised people include cytomegalovirus, adenovirus, invasive aspergillosis, Mycoplasma, Legionella, and Strongyloides. Influenza A (H1N1), dengue fever, leptospirosis, malaria, and Staphylococcus aureus infection are the most common infectious diseases that cause DAH in immunocompetent patients (18). Infection was implicated as the aetiology in ten of our cases, with two cases of Klebsiella pneumonia and one each of cytomegalovirus, Escherichia coli, tuberculosis, Candida, Aspergillus, mucormycosis, and Acinetobacter baumannii. All these cases were confirmed by microbiological studies. In one case, the causative agent could not be elucidated. One mortality seen in this group was due to mucormycosis.

Hemoptysis is a leading sign of DAH and may develop over days to weeks; however, it may be initially absent in up to one-third of DAH cases (6). The prevalence of hemoptysis is highly variable in different series, from 59.0% to 96.3%. Therefore, the absence of hemoptysis should not exclude this diagnosis (19). The symptoms of DAH, other than hemoptysis, are nonspecific and include fever, chest pain, cough, dyspnea, and respiratory failure. Non-pulmonary signs and symptoms accompany the underlying systemic disease (6). In the present study, hemoptysis was present in only 61.7% (n=29) of cases of DAH.

Typical pulmonary lesions of DAH on chest X-rays include bilateral, reticular, or nodular opacities. Chest computed tomography is indispensable in characterizing the pattern and extent of pulmonary disease (20). Ground-glass opacities and consolidation were the most common CT findings in our cases. Although MR has been used to confirm lung hemorrhage, it is probably of limited use in most clinical settings (9,21).

Our study had three cases of idiopathic pulmonary hemosiderosis (IPH) confirmed by lung biopsy. Idiopathic pulmonary hemosiderosis is a diagnosis

of exclusion and is an uncommon disease of childhood manifesting with the triad of recurrent hemoptysis, diffuse parenchymal infiltrates on chest radiographs, and iron deficiency anemia (22). The age of presentation is bimodal, with frequency peaks in children less than five years of age and adolescents 11 years or older (22). The diagnosis of IPH is based on the clinical picture, radiological findings on the chest X-ray and CT scan, demonstration of HLM in BALF, and exclusion of other diseases by appropriate serological, microbiological, and radiological tests (23). One case was of a 35-year-old female with Lane-Hamilton syndrome who presented with hemoptysis and, upon evaluation, was found to have chronic diarrhea with raised serum anti-tissue transglutaminase antibody levels. The duodenal biopsy revealed villous atrophy, BALF showed numerous HLMs, and a subsequent lung biopsy confirmed the findings of hemosiderosis (Fig 5).

Only 16 cases of Lane-Hamilton syndrome affecting adults have been reported in the literature thus far (24). Deposition of immunocomplexes (including food allergens) in the alveolar-capillary basement membrane and cross-reaction between antireticulin antibodies and alveolar basal membrane antigens are implicated in the pathogenesis (25,26).

In patients with evidence of DAH and renal involvement, a kidney biopsy may be considered to identify the etiology and guide the therapy (27). In a study by Fatma et al. on 15 cases of DAH associated with renal diseases, 13 underwent a kidney biopsy, of which ten showed crescentic pauci-immune glomerulonephritis (28). In our study, 11 out of 14 cases

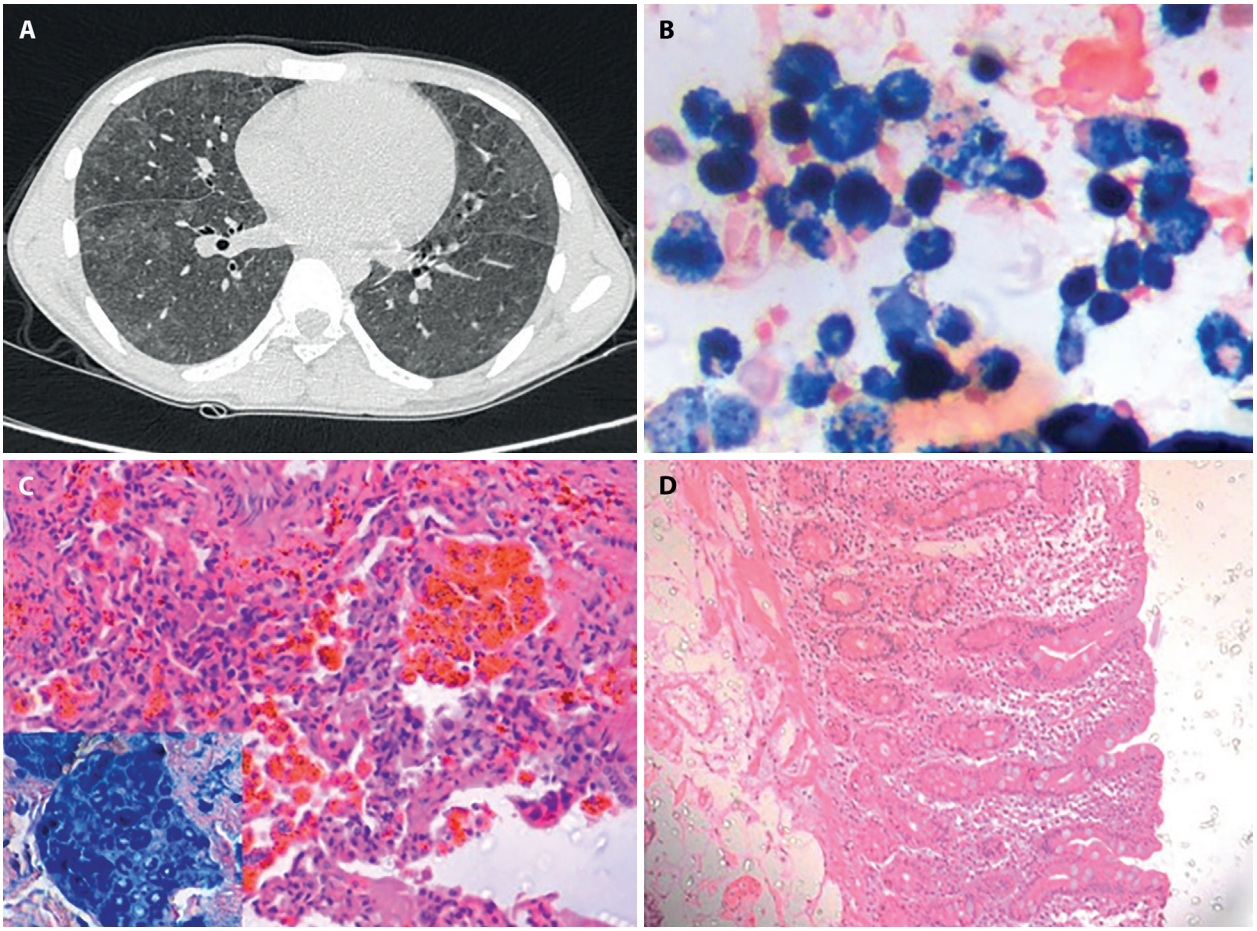

Figure 5. A: CT scan showing bilateral geographical ground-glass opacities. B: BAL fluid cytology smear showing numerous hemosiderophages containing bluish-green pigment (Perls' stain x200). C: Section from lung biopsy revealing aggregates of hemosiderin-laden macrophages, which stain positive for Perl's stain (inset) (H&E, x200). D: Section from duodenal biopsy displaying villous flattening and moderate inflammation in the lamina propria, suggestive of subtotal villous atrophy (H&E, x100) [Case of Lane Hamilton syndrome].

were diagnosed as crescentic glomerulonephritis, highlighting the utility of renal biopsy as an adjunctive tool in such cases.

Treatment of the cases was based on the underlying etiology. Patients with immune DAH were managed aggressively by immunosuppressive therapy, including steroids, cyclophosphamide, rituximab, intravenous immunoglobulins, and plasmapheresis. Established standard treatment regimens include corticosteroids and immunosuppressive therapy, but these can be detrimental when DAH is due to infection. Plasma exchange improves renal function in patients with ANCA-associated vasculitis. Rituximab can be used as an alternative or in addition to cyclophosphamide to treat AAV and SLE-associated DAH (12). In contrast, patients with nonimmune DAH were managed conservatively such as antibiotics and antifungals for infections. Hence, the prognosis for diffuse alveolar hemorrhage depends primarily on the classification proposed by us.

The main limitation of the study is that it is a retrospective study. Because the disease is rare, the sample size for proper calculation of risk factors is small, so the multivariate analysis may fail to give proper odds ratio in such a setting. A multicenter prospective study may throw more light on this disease.

Conclusion

DAH appears to be a heterogeneous syndrome because of varying etiologies. In our series, patients with immune DAH required more aggressive management. Female sex, erythrocyte sedimentation rate, and higher platelet count were significantly associated with immune-DAH. Finally, BALF cytology is an essential adjuvant investigation that can confirm a diagnosis of DAH in an appropriate clinical setting. Early recognition of DAH is crucial because prompt diagnosis and treatment are necessary for survival.

Conflict of Interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement, etc.) that might pose a conflict of interest in connection with the submitted article.

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