RESEARCH

Evaluation of hepatic toxicity in autoimmune hemolytic anemia (AIHA) and Evans syndrome patients: a single-center Egyptian study

Fatma Abozeid[1*] , Yasmine Shaaban[2] , Mohamed Elbogdady[2] and Esraa Jamal[2]

Abstract

Introduction Benign auto-immune illnesses include Evans syndrome (ES) and auto-immune hemolytic anemia (AIHA). Despite being benign in nature, the patients' livers are burdened by the disease's chronicity and the accompanying problems beyond the course of treatment. An additional burden stems from HCV infection, of which a significant proportion of Egyptians are positive. The purpose of this study was to identify the hepatotoxicity risks and the variables that influence the prognosis and survival of patients with AIHA/ES. There are 126 AIHA patients in this observational study, which is retrospective. From June 2009 to March 2021, patients visited the Haematology Unit of the Oncology Centre in Egypt. One hundred and sixteen patients have available data.

Results There was no significant difference between primary and secondary AIHA groups as regards baseline hemoglobin (Hb), bilirubin, LDH, or reticulocyte count. Thirty-four patients (29.31%) had HCV-positive tests and 1 patient (0.9%) had HBV. There was no difference between HCV-positive and negative cases as regards mean Hb concentration, mean platelet, or immune markers ($P > 0.05$). AIHA patients with HCV-positive showed a significantly higher relapse rate (56%) than HCV-negative patients (32%) ($P = 0.034$). HCV positivity and low platelet counts at diagnosis were poor predictors for overall survival (OS) (P 0.022 and 0.04, respectively). Median OS was significantly better in patients with no viral hepatitis infection (1101 days, 95% CI 592–2068) than in patients with positive HCV infection (521, 95% CI 326–1325) ($P = 0.019$).

Conclusions Azathioprine is the least hepatotoxic in AIHA patients under treatment. Viral hepatitis represents a superadded damage to the liver besides AIHA concerning clinical characteristics and outcomes.

Keywords AIHA, Evans syndrome, HCV, Azathioprine

Background

The term "auto-immune hemolytic anemia" (AIHA) refers to elevated erythrocyte turnover caused by immune system processes. One to three cases per 100,000 people is the estimated yearly incidence [1]. An autoimmune disorder known as Evans syndrome (ES) is characterized by the presence of two or more cytopenias, most commonly immune thrombocytopenia (ITP) and AIHA, either with or without immune neutropenia [2]. Based on the presence or absence of related diseases [3], such as systemic lupus erythematosus (SLE), lymphoproliferative disorders (LPD), malignancies, and drug-induced [2, 4], AIHA and Evan's syndrome are classified as main (idiopathic) or secondary disorders. Immune dysregulation or the production of neo-antigens can be brought on by medications and hematologic treatments such as hematopoietic stem cell transplantation (HSCT) and checkpoint inhibitors (CPI) [5]. The direct

*Correspondence:
Fatma Abozeid
Profatma2000@hotmail.com; Profatma2000@mans.edu.eg
[1] Gastroenterology and Hepatology (MD), Mansoura Specialized Medical Hospital, Mansoura University, Mansoura, Egypt
[2] Oncology Center of Mansoura University (OCMU), Mansoura University, Mansoura, Egypt

antiglobulin test (DAT), which allows the disease to be classified based on the isotype and thermal properties of the autoantibody, is the gold standard for the diagnosis of AIHA [6]. The appearance of reticulocytosis, unconjugated hyperbilirubinemia, decreased haptoglobin, and elevated LDH can all be indicators of hemolytic anemia. Since these signs are more common in liver illnesses, baseline examinations in patients with ES, AIHA, or both should include evaluations of the liver architecture, virology screening, and liver function testing in order to rule out or confirm the presence of hepatic affection. Hemolytic anemia may exhibit false-positive laboratory results in liver cell failure [7].

The etiology of AIHA is extremely intricate. Molecular mimicry, the formation of prohibited clones, polyclonal B cell activation and antibody production, and the disruption of T-lymphocyte homeostasis by antigen-presenting cells (APC) are the primary immunological mechanisms that lead to tolerance breakdown. Increased levels of interleukin (IL)-2, IL-4, IL-6, IL-10, and IL-12 are accompanied by a decrease in interferon-gamma (IFN-γ), which promotes differentiation, T-helper response, and humoral and cellular autoimmune. B cells interact with T-helper cells; extravascular hemolysis occurs when IgG autoantibodies are produced and induce destruction of the red blood cells (RBCs) through antibody-dependent cellular cytotoxicity and phagocytosis, primarily in the spleen; IgM autoantibodies strongly activate the complement system. Transforming growth factor beta (TGF-β) is increased leading to T-helper 17 differentiation and production of IL-17 that decreases T-regulatory levels. Extravascular hemolysis is caused by IgG autoantibodies, which destroy RBCs through antibody-dependent cellular cytotoxicity and phagocytosis, primarily in the spleen. On the other hand, IgM autoantibodies strongly activate the complement system, which destroys RBCs through C3b opsonization and phagocytosis, primarily in the liver. B cells interact with T-helper cells. IgM has the potential to cause terminal complement activation, which could result in intravascular hemolysis until the membrane assault complex forms. The effectiveness of the bone marrow compensatory response determines the degree of anemia [5].

In response to corticosteroids, primary warm-AIHA (w-AIHA) responds well. As a second-line treatment, splenectomy is effective in 70% of primary AIHA patients [8]. Rituximab is increasingly being used as the first-line treatment for cold agglutinin disease (CAD) and as the preferred second-line treatment for steroid-resistant w-AIHA [9]. Additional B cell targeting agents, such as obexelimab and ianalumab, are being investigated to lengthen the duration of response and lessen toxicity. Agents that target plasma cells, such as daratumumab

(ant-CD38 monoclonal antibody) and proteasome inhibitors like bortezomib, have demonstrated effectiveness in cases that are severe and refractory [10]. Immunosuppressants or the management of the underlying illness are two possible treatments for secondary AIHA, which is similar to how primary AIHA is treated [11].

The hepatitis virus's involvement in the start or progression of AIHA has not been extensively studied in reports. On the other hand, reports of hepatitis virus coinfection linked to AIHA are uncommon [12]. AIHA may be an uncommon extrahepatic sign of long-term HCV infection [13, 14]. Hemolytic anemia and acute hepatitis B rarely coexist [15]. In 25–50% of patients, it can cause subclinical hemolysis [16]. W-AIHA has been observed in hepatitis B virus carriers who do not exhibit any symptoms [17, 18]. Many medications, including azathioprine and androgens, which are used to treat AIHA and/or Evans syndrome, have varying degrees of hepatotoxicity and should be used cautiously in patients who have hepatic impairment [7].

The Egyptian Demographic and Health Surveys (EDHS) measured antibody prevalence among the adult population aged 15–59 years at 14.7% in 2009 and at 10.0% in 2015, significantly higher than the global levels estimated at 1.4% by the World Health Organization. Hepatitis C virus (HCV) prevalence in Egypt was estimated at 11.9% in a 2018 meta-analysis. As a result, 6 million people in Egypt have a chronic infection [19]. According to the WHO, HCV infection is a serious public health issue that affects 3% of the world's population and results in 2–3 million new cases annually [20]. According to estimates, 125,000 viremic individuals become infected with HCV annually in Egypt, where the prevalence rate of HCV infection was 872,000 in 2013 (15% of the population) [21].

The objectives of this study were to determine the liver status in patients with Evans syndrome and AIHA, evaluate the impact of various AIHA treatment modalities on liver function, and identify any potential influence of viral hepatitis on the clinical features and prognosis of AIHA.

Methods
From June 2009 to March 2021, 126 AIHA patients who visited the Haematology Unit at the Oncology Centre in Egypt were included in this retrospective observational study. There were 116 patients' worth of data. This study did not include patients with sickle cell disease, thalassemia, or hereditary spherocytosis, among other hereditary hemolytic disorders. Hepatocellular carcinoma, hypersplenism, and end-stage liver disease (Child score C) patients were not accepted. Patients with primary, secondary, and/or Evans syndrome who were 16 years of age or older and of either gender were eligible for this study.

Information was gathered from the registered medical records (https://srv137.mans.edu.eg/mus/newSystem/index.py) in accordance with the 1975 Helsinki Declaration, which was updated in 2008. The study was approved by the Faculty of Medicine's Ethical Committee (Code Number: RP.21.11.121).

Patients' complaints, medication histories for treating AIHA and/or Evans syndrome (corticosteroids, azathioprine, cyclosporine A, rituximab, splenectomy, etc.), and other medications that may cause secondary AIHA were among the information gathered for the presentation. Data from the physical examination were updated to include routine systemic examination for secondary cause detection as well as signs of hemolysis, such as jaundice, evaluation of comorbidities like diabetes, hypertension, or other conditions, and examination of the liver, spleen, and lymphoid regions.

In order to make a diagnosis, laboratory data were obtained at the patient's initial presentation, at 6 months, and after 6 months of starting therapy, as well as during their final follow-up appointment. Using an automated hematology analyzer, the following laboratory and radiological data were gathered: total leucocytic count, platelet count, and complete blood count (CBC). Total leucocytic, and platelets count using automated hematology analyzer Cell dyn 1700 and Cell dyn emerald hematology analyzer. The measuring unit for hemoglobin (Hb) was gm/dl, red cell count (RBC) was m/μL, total leucocytic count (TLC) was k/μL, and platelet count was k/μL. Laboratory data also included blood smear and reticulocyte count. Lactate dehydrogenase (LDH) (U/l) and Coomb's (direct and indirect).

Serum albumin (gm/dl), serum total bilirubin (0.3–1 mg/dl), direct and indirect bilirubin, aspartate aminotransferase (AST) (15–37 U/L), and alanine aminotransferase (ALT) (30–65 U/L) were among the tests used to estimate liver function using the Roche Cobas Integra-800 auto-analyzer. INR (International Normalised Ratio) as reported by Japan's Sysmex autoanalyzer. Virology markers, such as ELISA testing for HIV, HBV, and HCV. When possible, use an immune profile that includes ASMA, ANCA, anti-ds-DNA, and ANA to detect autoimmune hepatitis or SLE. For patients with concomitant thrombocytopenia, a bone marrow examination was performed to rule out lymphoproliferative disorders or underlying cancer. The radiological investigations consisted of a CT scan (of the chest, abdomen, and pelvis) to rule out solid tumors or lymphomas as hepatocellular carcinoma suspected for 2ry AIHA, as well as an abdominal ultrasound to comment on hepatic texture, spleen size, and the presence of ascites.

Treatment data included (according to our institutional protocols):

a. *Standard first-line* treatment was corticosteroids in the form of oral prednisone 1 mg/kg/day for at least 3 weeks then followed by gradual tapering or high dose dexamethasone (HDD) 40 mg intravenous (iv) for 4 days, or methylprednisolone (pulse steroid) iv 500–1000 mg/day for 3–5 days.

b. *As a second line*, treatment with rituximab (whenever available) at doses of 100 mg/m^2/week for 4 consecutive weeks or splenectomy. Oral azathioprine and cyclosporine A (CSA), mycophenolate mofetil (MMF), and others were also used as second or subsequent lines of treatment.

c. The response criteria were defined as follows [22]:

- Complete response (CR): normalization of hemoglobin with no evidence of hemolysis (normal bilirubin, LDH, and reticulocytes), and no need for transfusions.
- Response (R): increase in hemoglobin by > 2 g/dL or normalization of hemoglobin without biochemical resolution of hemolysis; and absence of transfusion.
- No response (NR): failure to achieve a response.
- Steroid resistance: failure to obtain a hematologic response within 3 weeks on at least 1 mg/kg prednisolone.
- Refractory disease failure to respond to at least 3 lines of therapy; in w-AIHA including splenectomy and/or at least one immunosuppressant.

d. Supportive blood/platelet transfusion once indicated:

Blood transfusion was done for any patient with hemoglobin < 7 g/dl and in cases of hemodynamic instability. While platelet transfusion was ordered once the platelet count ≤ 10,000 and in severe bleeding manifestations.

Statistical analysis

The statistical analysis and the visualization of the data were done using *R/R-Studio* (version 3.0) program. The data were read from Excel (Office program 2010). For the statistical analysis, different packages were used including "survival", "condsurv", "ggsigniff", "ggfortify", "Survminer", "rstatix", and "corrplot". For the visualization of the data, mainly the "tidyverse" package was used for the manipulation of the plots. The Shapiro–Wilk test was used to test the normality of the data. The qualitative and descriptive data were illustrated as numbers and percentages using the chi-square test. Quantitative data were analyzed as means, medians, and standard deviations. Wilcoxon test was used for the non-parametric data for the comparison between the two groups. ANOVA test was used for comparison between more than two non-parametric groups.

Abozeid *et al. The Egyptian Journal of Internal Medicine*   (2024) 36:22

Page 4 of 13

Also, Pearson correlation was used to correlate between different parameters. Kaplan–Meier test was used for the survival analysis. COX regression was used for univariate and multivariate analysis for the survival predictors. The significant *P* value was considered when less than 0.05.

Results

Baseline laboratory investigations of all patients are illustrated in Table 1 showing female predominance (71%) with a mean age of 43.3 ± 15 years. Most of the cases were primary AIHA (80.17%). Different autoimmune markers were assessed: direct Coomb's test (DCT) was positive in 99 patients. ASMA and ANCA tests were performed in 9 cases with suspected autoimmune hepatitis, and they were positive in only one patient.

There was no significant difference between both AIHA groups as regards baseline Hb levels ($P = 0.36$), platelet counts ($P = 0.13$), or response to therapy (Fig. 1).

HCV-positive patients showed a mean Hb concentration of 6.82 g/dL, mean platelet count of 176.53×10^9/L. There was no difference between HCV-positive and negative cases as regards mean Hb, mean platelets, LDH, or immune markers including (DCT, ANA, anti-ds DNA) ($P > 0.05$). While AIHA patients with HCV-positive status showed a significantly higher relapse rate (56%) than HCV-negative patients (32%) ($P = 0.034$).

Liver function follow-up in HCV-positive *vs.* HCV-negative cases at different milestones (at 1st presentation and then at follow-up intervals; 3–6 months after treatment, > 6 months from diagnosis, and at the last follow-up visits). There was a significant difference in SGOT level between the 2 studied groups when comparing the diagnosis to the last follow-up ($P = 0.0045$). A significant difference was also detected regarding the level of serum bilirubin between evaluated milestones.

A comparison of CBC parameters at the presentation and other time points is illustrated in Table 2. This comparison showed significant improvement in Hb levels from the baseline to the last follow-up evaluations rather than platelet or TLC.

It was found that, the mean SGPT levels showed no significant difference between patients who received or did not receive 2nd line therapy. On the other hand, the mean SGOT level and total bilirubin showed a statistically significant difference between the diagnosis levels and levels at the last follow-up in both groups ($P < 0.05$).

Median OS was significantly better in patients with no viral hepatitis infection (1101 days) than in patients with positive HCV infection (521 days) ($P = 0.019$).

In Table 3, patients with Hb levels ≤ 7 g/dL (severe anemia) at presentation had no significant difference in median OS than patients presenting with Hb levels > 7 g/dL ($P = 0.8$). While patients with platelet

Table 1 Clinical and laboratory characteristics of AIHA and/or ES studied patients $n = 116$

Characteristics	No. (%)
Age, years mean ± SD [range 16–72]	43.35 ± 15.30
Age of male patients, years mean ± SD [range 18–66]	44.68 ± 12.91
Age of female patients, years mean ± SD [range 16–72]	42.8 ± 16.24
Total patients' gender	
Male	34 (29.31)
Female	82 (70.69)
Evans syndrome (ES)	
Total	30 (25.86)
Male	10 (33.3)
Female	20 (66.7)
Aetiology of AIHA	
Primary	93 (80.17)
Secondary	23 (19.83)
Primary (females)	66 (56.9)
Primary (males)	27 (23.3)
Secondary (females)	16 (13.8)
Secondary (males)	7 (6.03)
Aetiology of secondary AIHA	
Autoimmune diseases	10 (43.5)
Viral hepatitis	5 (21.74)
Malignancy	4 (17.4)
Bone marrow disorders	3 (13.04)
Hashimoto thyroiditis	1 (4.35)
HCV infection status	
Positive	34 (29.31)
Negative	69 (59.48)
Unknown	13 (11.2)
Child–Pugh Score	
A	7 (6)
B	5 (4.3)
Complete blood count (CBC) at presentation mean ± SD	
TLC ($\times 10^9$/L)	10.36 ± 9.5
Hb (gm/dl)	6.78 ± 2.18
Platelet ($\times 10^9$/L)	177.86 ± 104.97
RC	12 ± 10.65
Liver function test (LFT) at presentation mean ± SD	
SGPT (U/L)	38.23 ± 39.53
SGOT (U/L)	57.45 ± 38.20
Total bilirubin (mg/dl)	3.99 ± 4.76
Direct bilirubin (mg/dl)	2 ± 3.67
Indirect bilirubin (mg/dl)	3.6 ± 3.48
Serum albumin (mg/dl)	3.77 ± 0.76
LDH	801.13 ± 893.31
Second line treatment	
Rituximab	16 (13.8)
Splenectomy	20 (17.24)
Azathioprine	46 (39.66)
Cyclosporine	20 (17.24)
Androgens	3 (2.6)

Table 1 (continued)

Characteristics	No. (%)
MMF	3 (2.6)
Combination of therapies	65 (56.03)
Immune markers	
DCT positive	99 (85.34)
DCT negative	14 (12.07)
DCT unknown	3 (2.6)
IDCT positive	79 (68.10)
IDCT negative	30 (25.9)
IDCT unknown	7 (6.03)
ANA positive	16 (13.8)
ANA negative	79 (68.1)
ANA unknown	21 (18.1)
Anti-ds-DNA positive	8 (6.9)
Anti-ds-DNA negative	75 (64.65)
Anti-ds-DNA unknown	33 (28.45)
ASMA positive	1 (0.9)
ASMA negative	7 (6.03)
ASMA unknown	108 (93.10)
ANCA positive	0
ANCA negative	9 (7.76)
ANCA unknown	107 (92.24)

AIHA autoimmune hemolytic anemia, *ES* Evans syndrome, *TLC* total leukocytes count, *Hb* hemoglobin, *RC* reticulocytic count, *SGOT* serum glutamic oxalo-acetic transaminase, *SGPT* serum glutamic pyruvic transaminase, *LDH* lactate dehydrogenase, *MMF* microfinolate, *DCT* direct Coomb's test, *IDCT* indirect Coomb's test, *ANA* anti-nuclear antibody, *ds-DNA* double-strand DNA, *ASMA* anti-smooth muscle antibody, *ANCA* anti-neutrophil cytoplasm antibody

counts $< 150 \times 10^9/L$ showed significantly shorter median OS than patients with normal counts (672 days *vs.* 1239 days)($P = 0.038$). There was no significant difference in median OS between corticosteroid responders and those who failed to respond to it ($P = 0.99$).

Tables 4, 5, and 6 show uni- and multivariate analysis.

HCV positivity and low platelet counts at diagnosis were poor predictors for the OS (P 0.022 and 0.04, respectively). Other factors such as age, gender, etiology, Evans syndrome, second-line intake, liver functions, hemoglobin level, Coomb's test status, LDH level, relapse, and refractoriness status did not impact the OS significantly.

The combinations with a significant impact on OS were platelet count with HCV, Direct Coomb's test, and second-line intake (P: 0.0048, 0.018, and 0.017 respectively).

Discussion

Benign auto-immune diseases include Evans syndrome and auto-immune hemolytic anemia. Even though these conditions are not cancerous, the fact that they are chronic and have accompanying problems—whether they are brought on by the illness or the therapy—makes them difficult for the patients who have them, particularly the hepatic complications. This study sought to identify the covariates influencing the survival and outcome of patients with AIHA/Evans syndrome, as well as the risks associated with hepatotoxicity, taking into account the additional burden on the liver from HCV infection, which is positive in a significant portion of the Egyptian population.

This is a retrospective study of 116 patients with a median age of 44 years (range, 16–72) which is slightly younger than that recorded in previous studies where the
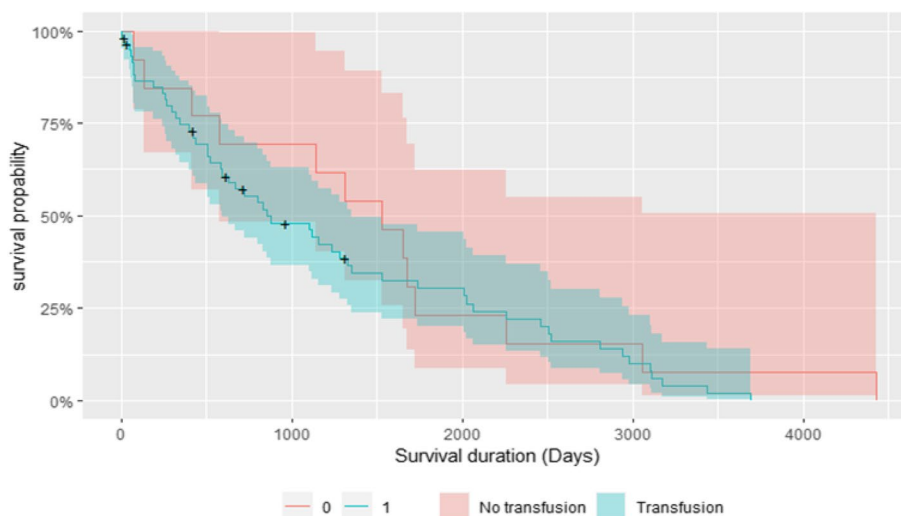

Fig. 1 PRBCs transfusion requirements. Patients who did not need PRBCs transfusion at any time of therapy = 25. Patients who needed PRBCs transfusion at any time of therapy = 87. Unknown = 4

Table 2 CBC parameters at different time points

Time point	(Mean ± SD) (Median; range)	Wilcoxon test *P* value
Hb conc		
At diagnosis	6.78 ± 2.2 6.5; 2–13	< 2.2e − 16
At last follow-up	10.28 ± 2.34 10; 4.9–15	
At diagnosis	6.55 ± 1.84 6.4; 3 − 11	1 and 2 = 0.0075 2 and 3 = 1.6e − 10
Before 2nd line (relapse)	7.3 ± 1.46 7; 3 − 10	1 and 3 = 3.6e − 13
At the last follow-up after 2nd or subsequent line	10.16 ± 2.3 10; 6 − 14	
PLT count		
At diagnosis	179.26 ± 104.32 178.35; 2.15 − 520	0.34
At last follow-up	203.51 ± 131.7 184; 3 − 655	
TLC		
At diagnosis	10.39 ± 9.53 7.45; 1.4 − 67	0.44
At last follow-up	8.87 ± 6.5 7.05; 2.2 − 48	

median ages were over 50 years [23, 24] which could be attributed to different sample sizes and older than what reported in a previous Indian study [25].

In this study (Table 1), there is a female predominance (71%) which is in agreement with different studies and reviews [26]. Most of our cases were primary AIHA (∼ 80%) with no obvious underlying etiology after performing the workup and ∼ 19% of the cases were secondary and 43.5% of them were related to autoimmune disease, e.g., SLE and RA (rheumatoid arthritis); this is corresponding to various previous studies [23, 24, 27]. Malignant hematological diseases were found in 17.4% of patients presenting with hemolysis. Bone marrow examination detected underlying hematological disorders in 4 patients [$n=1$ plasma cell dyscrasias, $n=1$ hairy cell leukemia (HCL), and $n=2$ Myelodysplastic syndrome]. CT scans revealed lymphadenopathy in 2 cases and were diagnosed as non-Hodgkin lymphoma (NHL).

Basma Atef et al. illustrated that the prevalence of AIHA, ITP (idiopathic thrombocytopenic purpura), and Evans syndrome was 6.9%, 3%, and 2% respectively among 101 chronic lymphocytic leukemia (CLL) patients in a previous observational retrospective study performed at our center [28].

Direct Coomb's test (DCT) was positive in 99 patients with immune cytopenia, and 14 patients had a negative test. A negative DCT test in warm autoimmune hemolytic anemia (w-AIHA) could be misleading and necessitates further evaluation. The most common cause of a negative test in AIHA is technical, other causes include erythrocyte-bound antibodies below the detection limit by standard tests, or the presence of low-affinity IgG, IgA, or IgM antibodies reacting at warm temperatures and monomeric IgM not fixing complement. The test may need to be repeated using anti-IgG and anti-C3d reagents. DCT-positive or DCT-negative w-AIHA have the same clinical criteria and response to therapy [1].

There was no significant difference between 1 ry and 2 ry AIHA groups as regards baseline hemoglobin, bilirubin, LDH, or reticulocyte count [29]. This is consistent with our study. Also, we did not even detect significance between them as regards age, gender, or response to therapy. AIHA 1ry *vs.* 2ry did not show a difference in response to corticosteroids [30] which is also seen in our results (Table 7).

Evans syndrome (ES) is rare (1–9 patients/1,000,000 people/year) and most data we get about it are from pediatric retrospective studies so less is understood about adults [31]. The incidence of ES among our

Table 3 Kaplan–Meier method in correlations with OS

	Events	Median OS (days)	Lower 95% CI	Upper 95% CI	*P* value	HR
Primary AIHA	55	856	574	1652	0.75	0.91
Secondary AIHA	14	1199	583	2208		
HCV-negative	42	1101	592	2068	**0.019**	**2**
HCV-positive	19	521	326	1325		
Hb level > 7 g/dl	23	1281	721	1677	0.8	0.94
Hb level ≤ 7 g/dl	46	799	506	2012		
Normal platelets ≥ 150 × 10⁹/L	43	1239	583	2023	**0.038**	**1.7**
Low platelets < 150 × 10⁹/L	26	672	506	1312		
Corticosteroid responsive	25	990	506	2068	0.99	1
Corticosteroid refractory	41	856	575	1720		

Bold is the significant results (as *P* less than 0.05)

Table 4 Univariate analysis (COX regression for predictors of OS)

Predictor	Beta	HR (95% CI for HR)	P value
Gender	0.055	1.1 (0.95–1.9)	0.85
Age	0.0098	1 (1–1)	0.19
Etiology	−0.099	0.91 (0.49–1.7)	0.75
Evans syndrome	0.24	1.3 (0.67–2.4)	0.47
HCV status	0.69	2 (1.1–3.6)	**0.022**
LDH	−0.03	0.97(0.57–1.6)	0.91
Hb level	−0.065	0.94 (0.56–1.6)	0.8
Platelet count	0.54	1.7(1–2.9)	**0.04**
DCT	0.43	1.5 (0.73–3.2)	0.26
IDCT	−0.039	0.96 (0.52–1.8)	0.9
Steroid response	0.0045	1 (0.61–1.7)	0.99
Second line	0.023	1 (0.61–1.7)	0.93
Relapse status	0.042	0.96 (0.58–1.6)	0.87
Refractory status	−0.25	0.78 (0.48–1.3)	0.3
Hepatic impairment	−0.04	0.96 (0.51–1.8)	0.9
PRBCs transfusion	0.24	1.3 (0.67–2.4)	0.47
SGPT	0.44	1.6 (0.76–3.2)	0.22
SGOT	0.12	1.1 (0.69–1.8)	0.63
T. Bilirubin	0.36	1.4 (0.77–2.7)	0.25

Bold is the significant results (as *P* less than 0.05)

Table 5 Multivariate analysis (COX regression for predictors of survival) (HCV with all the variables) (significant variables only)

Covariates	Beta	HR (95%CI for HR)	P value
HCV + gender	0.7	0.35 (1.1–2.6)	0.04
HCV + age	0.63	0.0066 (1–3.5)	0.051
HCV + platelets	0.78	0.66 (1.1–3.4)	0.0048
HCV + steroid response	0.74	−0.07 (0.53–1.6)	0.048
HCV + second line	0.69	0.023 (0.63–2)	0.024
HCV + relapse	0.78	−0.22(1.2–4.1)	0.054
HCV + refractory status	0.63	−0.39 (1–3.4)	0.025

Table 6 Multivariate analysis (COX regression for predictors of survival) (platelets with all the variables) (significant variables only)

Covariates	Beta	HR (95%CI for HR)	P value
Platelets + HCV	0.66	0.78 (1.1–3.4)	0.0048
Platelets + DCT	0.73	0.74 (1.2–3.6)	0.018
Platelets + second line	0.65	0.023 (1.1–3.3)	0.017

Table 7 Comparison between different parameters in 1ry and 2ry AIHA

	1ry AIHA (*n* = 93)	2ry AIHA (*n* = 23)	P value
Patients' classification based on different parameters number (%)			
Female	66 (71%)	16 (69.6%)	1
Male	27 (29.03%)	7 (30.43%)	
Non relapsed	55 (59.14%)	15 (65.2%)	0.77
Relapsed	38 (41%)	8 (35%)	
Non-refractory	49 (52.7%)	12 (52.17%)	1
Refractory	44 (47.3%)	11 (48%)	
Laboratory parameters (mean ± SD) (Median; range)			
Hb concentration	6.7 ± 2.2	7 ± 2.15	
	(6.4; 2–13)	(7; 3–10)	0.36
Platelet count	186.51 ± 107.68	149.93 ± 85.28	
	(182; 5–520)	(143; 2.15–340)	0.13

international retrospective study showed that age, baseline Hb level < 8 g/dL, and occurrence of relapse, infection, and thrombosis were poor prognostic indicators of survival [31]. In our ES cases, we did not find a correlation with age, HCV status, response to 1st line, relapse, or refractoriness to further treatments. ES diagnosis did not affect survival, these differences between our results and the international multicenter study could be attributed to their larger sample size and their focus on ES characteristics.

Viral infections have been observed with AIHA, e.g., human immune deficiency virus (HIV), Epstein-Barr virus (EBV), cytomegalovirus (CMV), Parvovirus B19, and HCV. Viral infections trigger autoimmunity by mimicking molecular pathways between self-antigens and foreign viral antigens. The first International Consensus Meeting recommended testing for HBV, HCV, and HIV at baseline for patients presenting with AIHA and before initiation of therapy, while other viruses like EBV, CMV, and Parvovirus B19 are requested under certain circumstances [4, 22, 32]. Viral hepatitis is capable of inducing extrahepatic immune dysregulation and affection leading to immune cytopenia as AIHA [33]. HCV-associated AIHA has been described before in research as case reports or series [13, 34], while other reports have attributed hemolytic anemia to antiviral hepatitis drugs [35, 36].

In our study, 34 patients (29.31%) had HCV-positive tests, 1 patient (0.9%) had HBV positive test, and no one was HIV-positive at diagnosis, while virology data were missing in 13 patients. A previous study showed that the incidence of AIHA among Egyptian patients with chronic HCV was 0.7% [37]. In another Egyptian study, AIHA

studied patients was 26% and 67% of them were females with a mean age of 40 years and the incidence of positive HCV test among them was 26% (7 patients). The ES

Abozeid *et al. The Egyptian Journal of Internal Medicine*　　(2024) 36:22

Page 8 of 13

Table 8 Comparison between HCV-positive and HCV-negative AIHA and/or Evans syndrome patients

Comparison parameters	HCV-positive patients ($n = 34$)	HCV-negative patients ($n = 69$)	P value
Patients' classification based on etiology numbers (%)			
AIHA patients	27 (79.41%)	49 (71.01%)	0.5
Evans syndrome patients	7 (20.5%)	20 (29%)	
Patients' classification based on response to treatment numbers (%)			
Relapsed patients	19 (56%)	22 (32%)	0.034
Not relapsed patients	15 (44.11%)	47 (68.11%)	
Refractory patients	11 (32.4%)	40 (58%)	0.025
Not refractory patients	23 (68%)	29 (42%)	
Hepatic impairment after therapy	12 (39%)	7 (10.14%)	0.0025
No hepatic impairment after therapy	19 (61.3%)	60 (89.6%)	
Patients' classification based on immune profile numbers (%)			
Patients with positive DCT (total $n = 99$)	32 (94.11%)	57 (82.6%)	0.31
Patients with negative DCT (total $n = 14$)	2 (5.9%)	10 (14.5%)	
Patients with ANA-negative	21 (61.76%)	50 (72.5%)	0.29
Patients with ANA-positive	6 (17.6%)	6 (8.7%)	
Patients with anti-ds-DNA negative	19 (56%)	46 (66.7%)	1
Patients with anti-ds-DNA positive	2 (5.8%)	4 (5.8%)	
Laboratory parameters (Mean ± SD) (Median; range)			
LDH	739.55 ± 735.98	846.1 ± 958.81	
	(504; 100–3763)	(511;157–4818)	0.42
Hb concentration	6.82 ± 2.09	6.72 ± 2.2	
	(6.16; 3.5–12.76)	(6.6; 2–13)	0.94
Platelet count	176.53 ± 81.33	177.93 ± 115.67	
	(176.35; 10–348)	(165; 5–520)	0.9

was diagnosed in only 2 (0.95%) HCV patients, and 1 of them was induced by anti-viral therapy [38].

In Table 8, there was no difference between HCV-positive and negative cases as regards mean Hb concentration, mean PLT, or immune markers ($P > 0.05$). Previously 17 HCV-positive AIHA patients (F/M: 15/2; mean age, 55.8 years) were studied; their mean Hb concentration was 7.1 g/dL (range, 2.8–10.0 g/dL). ANA and anti-ds-DNA represented 41% and 6%, respectively of their immune profile [39].

Concerning the treatment protocol, all patients received corticosteroids as first-line treatment of choice for 1ry AIHA and/or Evans syndrome and in combination with the definitive therapy for 2ry AIHA patients with treatable causes. The steroid responders were estimated as the patients who achieved complete response (CR) or response (R) [$n = 48$ patients (41.38%)], and the non-responders (NR) were estimated as the patients who were steroid resistant [$n = 65$ patients (56.03%)], there were 3 patients with unknown response to

steroids as they lost follow-up. Approximately 62.93% of the total included patients received second-line treatments.

To define the correlation between HCV status and the response to steroids, the chi-square test was used revealing that there is a positive correlation between HCV positivity and steroid response but with no statistical significance ($P = 0.13$) (see Table 8). AIHA patients with HCV-positive status showed a significantly higher relapse rate (56%) than HCV-negative patients (32%) ($P = 0.034$). On the other hand, AIHA patients with HCV-negative status showed a higher treatment refractoriness or resistance rate (57%) than HCV-positive patients (32%) ($P = 0.025$). Hepatic impairment after treatment was more pronounced in HCV-positive patients (39%) than HCV-negative patients (10%) ($P = 0.002$), which indicates a higher burden of HCV on these patients.

The significant difference that was found in SGOT level (rather than SGPT) between the 2 studied groups when comparing the diagnosis to the last follow-up ($P < 0.05$),

Table 9 Liver functions in HCV-positive vs. HCV-negative cases at different milestones ($n = 103$)

Liver functions median (range)	1)Diagnosis	2) < 6 m of FU	3) > 6 m of FU	4)At last FU	ANOVA test
SGPT					
HCV +	31.5 (10–407)	43 (11–623)	31 (4–219)	31 (8–311)	*P* values:
(HCV −)	25 (6–772)	27 (8–177)	21 (8–334)	24 (6–315)	1:2 = 0.5
					1:3 = 0.22
					1:4 = 0.22
SGOT					
HCV +	43 (8–284)	50.5 (10–387)	49 (10–172)	36 (6–390)	*P* values:
(HCV −)	42 (10–492)	27.5 (9–194)	28 (12–211)	26 (10–650)	1:2 = 0.26
					1:3 = 0.17
					1:4 = 0.0045
Total bilirubin					
HCV +	3.45 (0.61–26.7)	1.2 (0.4–26)	1.3 (0.5–12)	1.3 (0.4–22)	*P* values:
(HCV −)	2.5 (0.2–30)	1 (0.2–10)	1.3 (0.1–16)	0.9 (0.1–7)	**1:2 = 3.3e − 05**
					1:3 = 6e − 05
					1:4 = 2e − 09

as well as, in the level of serum bilirubin between evaluated milestones could be related to the super added effect of HCV on liver condition besides the drug-induced liver injury caused by different lines of treatment especially cyclosporine (see Table 9).

The liver enzymes and total bilirubin were compared in patients who received 2nd line therapy to patients who did not receive 2nd line. The patients were compared as a total and then sub-classified according to the type of the 2nd line received. The mean SGOT level and bilirubin (rather than SGPT) showed a statistically significant difference between the diagnosis levels and levels at the last follow-up in both groups. This can be attributed to the cholestatic pattern of liver injury induced by these drugs. To dissect the previous data and go into its depth, the enzymes, and total bilirubin were analyzed regarding each 2nd line therapy used.

In patients who received rituximab, there was no significant difference in the mean SGPT or SGOT levels between the diagnosis and any time point in contrast to the patients who did not receive rituximab who showed a significant difference in the mean of the enzymes' levels between diagnosis and last follow-up. However, the total bilirubin showed a significant difference in the means at diagnosis and the last follow-up in patients who received rituximab. In patients who received azathioprine, SGPT levels did not show any significant differences between diagnosis and different time points. SGOT levels showed significant differences between diagnosis and last follow-up in patients who did not receive azathioprine. Total bilirubin showed significant differences between diagnosis and all the time points in both groups. Serum bilirubin

showed a significant difference between the time of diagnosis and all time-point levels in the arm of patients who didn't receive cyclosporine and only a significant difference between the diagnosis and last follow-up levels in patients who received cyclosporine. The same significant differences were seen when we assessed the patients who received combined therapies as in Table 10.

These results indicate that liver functions are improving better in patients who did not receive 2nd line therapy. SGOT and bilirubin are more sensitive to hemolysis status and drug toxicity. Based on the results, the least hepatotoxic drug that could be considered is azathioprine as the liver functions improved between the diagnosis and the last follow-up in both groups.

According to survival, there was no significant difference in the overall survival (OS) regarding the gender of studied patients; the median OS for male patients was 839 days (95% CI 506–1743) and the median OS for female patients was 1101 days (95% CI 583–1720) ($P = 0.86$). There was also no significant difference in OS between 1ry or 2ry cases ($P = 0.75$). However, median OS was significantly better in patients with no viral hepatitis infection (1101 days, 95% CI 592–2068) than in patients with positive HCV infection (521, 95% CI 326–1325) ($P = 0.019$) (Fig. 2).

HCV positivity and low platelet counts at diagnosis were found to be poor predictors for the OS (*P* 0.022 and 0.04, respectively). Multi-factorial combinations were analyzed by combining them using COX regression. A negative impact on OS with statistical significance was detected between HCV status with gender, HCV status with age, HCV status with platelet counts,

Table 10 Comparison between patients who received and did not receive different Second-line therapies regarding liver functions (SGPT, SGOT, and total bilirubin)

Time points	Mean	Median	Range	SD	Wilcoxon test *P* value
1) SGPT					
Received the second line					
1)Diagnosis	58.31	28.8	6–772	109.65	1 and 2 = 0.78
2) < 6 m	55.96	30	11–623	89.90	1 and 3 = 0.43
3) > 6 m	44.51	26.5	4–334	55.95	1 and 4 = 0.49
4)Last follow-up	45.85	26	6–343	60.83	
Did not receive the second line					
1)Diagnosis	34.18	30	4.5–134	25.02	5 and 6 = 0 .56
2) < 6 m	43.54	30	8–130	36.19	5 and 7 = 0.17
3) > 6 m	29.37	20	10–91	22.01	5 and 8 = 0.064
4)Last follow-up	25.33	19	8–79	16.72	
Rituximab					
1)Diagnosis	24.36	20.53	6–54	14.8	1 and 2 = 0.49
2) < 6 m	75.07	20	11–623	155.2	1 and 3 = 0.94
3) > 6 m	27.08	19.5	4–90	23.53	1 and 4 = 0.44
4)Last follow-up	45.33	26	9–311	74.83	
No rituximab					
1)Diagnosis	53.78	30	4.5–772	94.36	1 and 2 = 0.67
2) < 6 m	48	30.5	8–289	48.39	1 and 3 = 0.1
3) > 6 m	40.89	22	8–334	49.31	**1 and 4 = 0.053**
4)Last follow-up	41.12	24	6–343	55.6	
Azathioprine					
1)Diagnosis	71.12	29	8–772	133.05	1 and 2 = 0.63
2) < 6 m	49.83	28	11–289	58.95	1 and 3 = 0.14
3) > 6 m	37.45	25	4–219	40.95	1 and 4 = 0.076
4)Last follow-up	38.35	24	6–343	56.06	
No azathioprine					
1)Diagnosis	35.38	28.3	4.5–159	29.02	1 and 2 = 0.2
2) < 6 m	54.35	31	8–623	86.93	1 and 3 = 0.48
3) > 6 m	40.14	21	10–334	50.97	1 and 4 = 0.59
4)Last follow-up	44.19	24.5	7–315	60.88	
Combination					
1)Diagnosis	60.40	28.8	6–772	114.5	1 and 2 = 0.76
2) < 6 m	58.10	29.5	11–623	93.71	1 and 3 = 0.37
3) > 6 m	45.01	25.5	4–334	57.91	1 and 4 = 0.57
4)Last follow-up	47.54	26	6–343	63.79	
No combination					
1)Diagnosis	36.99	30	4.5–159	29.45	1 and 2 = 0.73
2) < 6 m	44.52	30.5	8–172	39.44	1 and 3 = 0.13
3) > 6 m	30.24	20	10–91	21.37	**1 and 4 = 0.031**
4)Last follow-up	25.79	19	8–79	16.29	

Table 10 (continued)

Time points	Mean	Median	Range	SD	Wilcoxon test *P* value
2)SGOT					
Received 2nd line					
1)Diagnosis	64.39	39	8–492	86.35	1 and 2 = 0.36
2) < 6 m FU	55.89	33	10–387	70.12	1 and 3 = 0.3
3) > 6 m FU	45.15	29	10–211	38.71	**1 and 4 = 0.026**
4)Last FU	44.58	29	6–390	59.68	
Did not receive 2nd line					
1)Diagnosis	45.59	40	12–168	32.09	1 and 2 = 0.54
2) < 6 m FU	45.64	29.5	9–189	42.08	1 and 3 = 0.56
3) > 6 m FU	42.48	34	12–172	34.76	**1 and4 = 0.031**
4)Last FU	41.65	20.5	10–261	57.76	
Rituximab					
1)Diagnosis	37.02	34.93	8–101	23.71	1 and2 = 0.4
2) < 6 m FU	56.15	21	10–387	102.05	1 and3 = 0.68
3) > 6 m FU	32.92	26.5	10–66	19.89	1 and 4 = 0.65
4)Last FU	54.86	31	6–390	97.61	
No rituximab					
1)Diagnosis	60.43	39.5	10–492	76.21	1 and 2 = 0.35
2) < 6 m FU	50.60	32	9–274	50.13	1 and 3 = 0.3
3) > 6 m FU	45.89	30	12–211	38.72	**1 and 4 = 0.0018**
4)Last FU	49.08	24.5	10–650	85.97	
Azathioprine					
1)Diagnosis	73.97	33.78	10–492	107.87	1 and 2 = 0.35
2) < 6 m FU	49.13	28	10–274	56.94	1 and 3 = 0.58
3) > 6 m FU	45.96	28	13–211	44.99	1 and 4 = 0.11
4)Last FU	37.97	29	6–198	38.93	
No azathioprine					
1)Diagnosis	46.96	40	8–175	32.5	1 and 2 = 0.46
2) < 6 m FU	53.28	31.5	9–387	64.89	1 and 3 = 0.39
3) > 6 m FU	42.46	36	10–172	30.01	**1 and 4 = 0.0077**
4)Last FU	57.90	21.5	10–650	107.63	
Cyclosporine					
1)Diagnosis	40.39	35.43	10–87	23.79	1 and 2 = 0.77
2) < 6 m FU	43.98	27.5	12–180	44.26	1 and 3 = 0.73
3) > 6 m FU	49	33.5	12–160	40.27	1 and 4 = 0.46
4)Last FU	40.76	28	12–198	43.87	
No cyclosporine					
1)Diagnosis	61.03	39.74	8–492	78.57	1 and 2 = 0.25
2) < 6 m FU	53.67	30	9–387	65.71	1 and 3 = 0.17
3) > 6 m FU	43.17	28.5	10–211	36.57	**1 and 4 = 0.0016**
4)Last FU	43.51	22.5	6–390	61.55	
Combined therapies					
1)Diagnosis	64.74	37.43	8–492	91.06	1 and 2 = 0.59
2) < 6 m FU	57.99	32	10–387	72.82	1 and 3 = 0.38
3) > 6 m FU	44.53	28	10–211	40.16	1 and 4 = 0.084
4)Last FU	45.94	29	6–390	62.62	

Abozeid *et al. The Egyptian Journal of Internal Medicine*   (2024) 36:22

Page 11 of 13

Table 10 (continued)

Time points	Mean	Median	Range	SD	Wilcoxon test P value
No combined therapies					
1)Diagnosis	48.06	40	12–175	35.41	1 and 2 = 0.18
2)<6 m FU	42.02	29	9–189	38.8	1 and 3 = 0.69
3)>6 m FU	44.59	39	12–172	32.68	**1 and 4 = 0.0035**
4)Last FU	39.06	21	10–261	52.2	
3)Total bilirubin					
Received 2nd line therapy					
1)Diagnosis	3.72	2.9	0.2–30	4.18	1and **2 = 0.0002**
2)<6 m FU	2.58	1.3	0.3–26	3.78	**1 and 3 = 0.00029**
3)>6 m FU	2.62	1.46	0.2–16	3.25	**1 and 4 = 1.2e − 08**
4)Last FU	2	1	0.1–22	3.47	
Did not receive 2nd line					
1)Diagnosis	3.6	2.1	0.2–26.7	5.06	**1 and 2 = 0.008**
2)<6 m FU	1.7	0.9	0.2–12.5	2.57	**1 and 3 = 0.0078**
3)>6 m FU	1.45	0.95	0.1–6.2	1.39	**1 and 4 = 0.001**
4)Last FU	1.27	0.7	0.1–54	1.54	
Rituximab					
1)Diagnosis	4.55	3.58	0.7–21	4.84	1 and 2 = 0.2
2)<6 m FU	4.25	2.5	0.3–26	6.74	1 and 3 = 0.24
3)>6 m FU	3.08	1.5	0.5–12	3.39	**1 and 4 = 0.003**
4)Last FU	1.39	1	0.1–5.7	1.35	
No rituximab					
1)Diagnosis	3.5	2.7	0.2–30	4.36	**1 and 2 = 4.5e − 06**
2)<6 m FU	1.85	1.1	0.2–12.5	2.05	**1 and 3 = 7.2e − 06**
3)>6 m FU	2.06	1.15	0.1–16	2.71	**1 and 4 = 1.1e − 08**
4)Last FU	1.84	1	0.1–22	3.18	
Azathioprine					
1)Diagnosis	3.77	3	0.2–30	4.4	**1 and 2 = 0.00015**
2)<6 m FU	1.88	1.2	0.4–10	1.85	**1and3 = 0.0011**
3)>6 m FU	2.27	1.4	0.2–16	2.85	**1 and 4 = 9.3e − 05**
4)Last FU	2.48	1.1	0.3–22	4.39	
No Azathioprine					
1)Diagnosis	3.6	2.5	0.2–26	4.47	**1 and 2 = 0.0034**
2)<6 m FU	2.53	1.05	0.2–26	4.17	**1 and 3 = 0.00086**
3)>6 m FU	2.17	1.1	0.1–15	2.82	**1 and 4 = 1.9e − 07**
4)Last FU	1.31	0.9	0.1–7	1.32	
Cyclosporine					
1)Diagnosis	3.05	2.9	0.7–7.2	2.15	1 and 2 = 0.25
2)<6 m FU	2.17	2	0.3–4.8	1.52	1 and 3 = 0.55
3)>6 m FU	3.17	1.5	0.2–16	3.71	**1 and 4 = 0.022**
4)Last FU	2.34	0.9	0.1–17	4.03	
No cyclosporine					
1)Diagnosis	3.81	2.85	0.2–30	4.78	**1 and 2 = 9.4e − 06**
2)<6 m FU	2.32	1.1	0.2–26	3.75	**1 and 3 = 7.6e − 07**
3)>6 m FU	1.97	1.2	0.1–15	2.54	**1 and 4 = 4.6e − 10**
4)Last FU	1.6	1	0.1–22	2.7	

Table 10 (continued)

Time points	Mean	Median	Range	SD	Wilcoxon test P value
Combined therapies					
1)Diagnosis	3.76	2.95	0.2–30	4.4	**1 and 2 = 0.0011**
2)<6 m FU	2.65	1.3	0.3–26	3.98	**1 and 3 = 0.001**
3)>6 m FU	2.65	1.4	0.2–16	3.39	**1 and 4 = 2.1e − 07**
4)Last FU	2.05	1	0.1–22	3.71	
No combined therapies					
1)Diagnosis	3.55	2.45	0.2–26.7	4.54	**1 and 2 = 0.0019**
2)<6 m FU	1.75	0.9	0.2–12.5	2.32	**1 and 3 = 0.00088**
3)>6 m FU	1.49	1	0.1–6.2	1.37	**1 and 4 = 7.5e − 05**
4)Last FU	1.34	0.8	0.1–7	1.42	

and HCV with second-line intake (P 0.04, 0.051, 0.0048, and 0.024, respectively). Other HCV combinations may have a significant protective impact on the OS of AIHA patients such as HCV status with steroid response status, and HCV with relapse or refractoriness status (P 0.048, 0.054, and 0.025 respectively) as shown in Table 5.

We did not find a relation between response to corticosteroids and baseline Hb or platelet levels or HCV status. Abdallah et al. and Barcellini et al. agreed that baseline Hb level had a negative predictive effect on response [40], while in our study there was no impact of baseline Hb concentration on OS (P 0.8), and it was the platelet count $< 150 \times 10^9$/L that had a negative impact on OS as shown by Kaplan–Meier, univariate and multivariate COX regression analyses.

Limitations facing our study included its retrospective nature, the sample size, and the lack of uniformity during second-line management. We faced difficulties in analyzing relapses and refractoriness, and collecting liver function data during follow-up because there are no uniform guidelines for choosing a drug over the other, we usually face problems with drug availability financially and on the other hand the patient's affordability and tolerability to treatments.

However, there was an analysis of quite a large number of patients including the section of patients with Evans syndrome. Also, we compared primary and secondary AIHA as well as HCV-positive and HCV-negative patients receiving different lines of treatment.

In conclusion, there is impairment of hepatic status either clinical (more decompensation) or laboratory parameters (SGOT, SGPT, and serum bilirubin) in patients with AIHA and Evans syndrome. The effect of different lines of treatment of AIHA on liver enzymes is variable and it needs meticulous follow-up, especially for
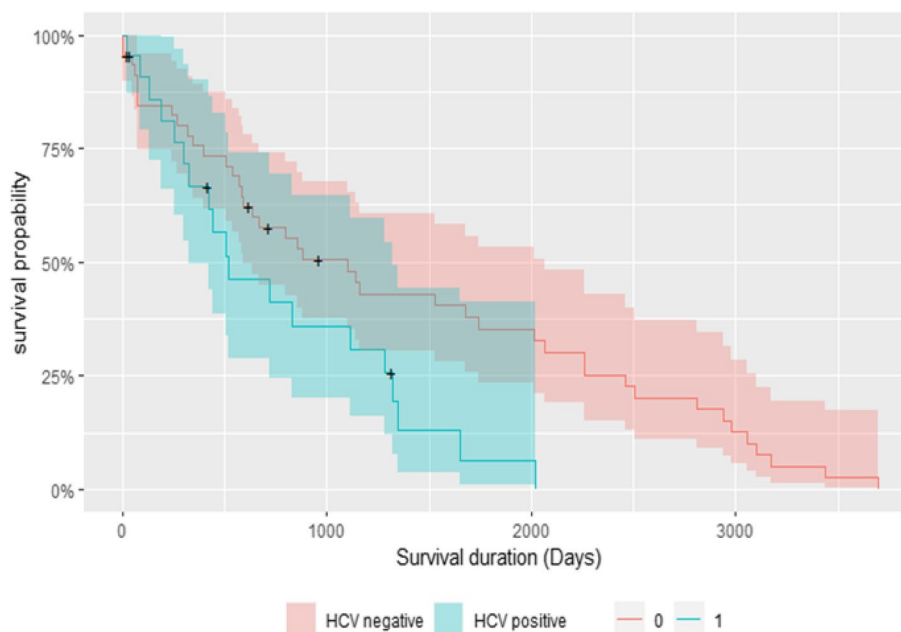
Fig. 2 Survival probability between HCV-positive and HCV-negative cases

serum bilirubin. The least hepatotoxic drug observed was azathioprine. Viral hepatitis infection (especially HCV) represents a superadded damage to the liver besides AIHA concerning clinical characteristics and outcomes. Median OS was significantly better in patients with no viral hepatitis infection ($P=0.019$).

Follow-up liver enzymes and serum bilirubin are a must when we shift the treatment from one line to another and once suspected hepatic adverse events. Larger prospective studies are needed to validate the results.

Abbreviations

AIHA	Autoimmune hemolytic anemia
ES	Evan syndrome
TLC	Total leukocytes count
Hb	Hemoglobin
RC	Reticulocyte count
SGOT	Serum glutamic oxalo-acetic transaminase
SGPT	Serum glutamic pyruvic transaminase
LDH	Lactate dehydrogenase
MMF	Mycophenolic
DCT	Direct Coomb's test
IDCT	Indirect Coomb's test
ANA	Anti-nuclear antibody
ds-DNA	Double-strand DNA
ASMA	Anti-smooth muscle antibody
ANCA	Anti-neutrophil cytoplasm antibody

Authors' contributions
Fatma Abozeid analyzed the data, and wrote and revised the manuscript. Yasmine Shaaban collected and revised the data, and wrote the manuscript. Mohamed Elbogdady collected and revised the data. Esraa Jamal collected the data and statistical analysis. All authors read and approved the final manuscript.

Declarations

Ethics approval and consent to participate
The Ethical Committee of the Faculty of Medicine of Mansoura University approved the study and the patients to participate (Code Number: RP.21.11.121).

Consent for publication
All authors have confirmed the manuscript for submission and publication in this journal.

Competing interests
The authors declare that they have no competing interests.

References
1. Go RS, Winters JL, Kay NE (2017) How I treat autoimmune hemolytic anemia. Blood J Am Soc Hematol 129(22):2971–2979
2. Shaikh H, Mewawalla P. Evans syndrome. StatPearls. 2021.
3. Zanella A, Barcellini W (2014) Treatment of autoimmune hemolytic anemias. Haematologica 99(10):1547–1554
4. Barcellini W, Fattizzo B (2020) The changing landscape of autoimmune hemolytic anemia. Front Immunol 11:946
5. Fattizzo B, Barcellini W (2022) Autoimmune hemolytic anemia: causes and consequences. Expert Rev Clin Immunol 18(7):731–745

6. Hill QA, Stamps R, Massey E, Grainger JD, Provan D, Hill A et al (2017) The diagnosis and management of primary autoimmune haemolytic anaemia. Br J Haematol 176(3):395–411

7. Barcellini W, Fattizzo B (2021) How I treat warm autoimmune hemolytic anemia. Blood J Am Soc Hematol 137(10):1283–1294

8. Hill A, Hill QA (2018) Autoimmune hemolytic anemia. Hematology 2018(1):382–389

9. Fattizzo B, Zaninoni A, Pettine L, Cavallaro F, Di Bona E, Barcellini W (2019) Low-dose rituximab in autoimmune hemolytic anemia: 10 years after. Blood 133(9):996–998

10. Cavallaro F, Barcellini W, Fattizzo B. Antibody based therapeutics for auto-immune hemolytic anemia. Expert Opin Biol Ther. 2023 (just-accepted).

11. Lechner K, Jäger U (2010) How I treat autoimmune hemolytic anemias in adults. Blood 116(11):1831–1838

12. Jia L-j, Zhang J-b, Li W-m, Bo Y-k (2014) HBV and HCV coinfection associ-ated with warm-type autoimmune hemolytic anemia: a case report/HBV ve HCV Koenfeksiyonu ile Iliskili Sicak Tip Otoimmün Hemolitik Anemi: Bir Olgu Sunumu. Turk J Haematol 31(3):328

13. Basseri RJ, Schmidt MT, Basseri B (2010) Autoimmune hemolytic anemia in treatment-naive chronic hepatitis C infection: a case report and review of literature. Clin J Gastroenterol 3(5):237–242

14. Elhajj II, Sharara AI, Taher AT (2004) Chronic hepatitis C associated with Coombs-positive hemolytic anemia. Hematol J 5(4):364–366

15. Vanderhoof J, Ament M (1976) Severe Coombs negative hemolytic anemia in hepatitis B. West J Med 125(3):228

16. Conrad ME (1969) Persistent haemolysis after infectious hepatitis. Gut 10(7):516

17. Yoshioka K, Miyata H (1980) Autoimmune haemolytic anaemia in an asymptomatic carrier of hepatitis B virus. Arch Dis Child 55(3):233–234

18. Khawaja S, Abdul MK, Taj Y (2011) Warm autoimmune haemolytic anae-mia and autoimmune hepatitis in an asymptomatic carrier of hepatitis B virus. JPMA J Pakistan Med Assoc 61(5):512

19. Anwar WA, El Gaafary M, Girgis SA, Rafik M, Hussein WM, Sos D et al (2021) Hepatitis C virus infection and risk factors among patients and health-care workers of Ain Shams University hospitals, Cairo, Egypt. Plos one 16(2):e0246836

20. Organization WH. Guidelines for the screening, care and treatment of persons with hepatitis C infection: World health organization; 2014.

21. Waked I, Doss W, El-Sayed MH, Estes C, Razavi H, Shiha G et al (2014) The current and future disease burden of chronic hepatitis C virus infection in Egypt. Arab J Gastroenterol 15(2):45–52

22. Jäger U, Barcellini W, Broome CM, Gertz MA, Hill A, Hill QA et al (2020) Diagnosis and treatment of autoimmune hemolytic anemia in adults: recommendations from the First International Consensus Meeting. Blood Rev 41:100648

23. Jomarrón IG, Rubio ML, Arias MM, Arrizabalaga B, de la Iglesia S, Beneitez D et al (2020) Autoimmune haemolytic anaemias: a retrospective study of 93 patients. Medicina Clínica (English Edition) 154(9):331–337

24. Roumier M, Loustau V, Guillaud C, Languille L, Mahevas M, Khellaf M et al (2014) Characteristics and outcome of warm autoimmune hemolytic anemia in adults: new insights based on a single-center experience with 60 patients. Am J Hematol 89(9):E150–E155

25. Naithani R, Agrawal N, Mahapatra M, Pati H, Kumar R, Choudhary V (2006) Autoimmune hemolytic anemia in India: clinico-hematological spectrum of 79 cases. Hematology 11(1):73–76

26. Bass GF, Tuscano ET, Tuscano JM (2014) Diagnosis and classification of autoimmune hemolytic anemia. Autoimmun Rev 13(4–5):560–564

27. Dierickx D, Kentos A, Delannoy A (2015) The role of rituximab in adults with warm antibody autoimmune hemolytic anemia. Blood J Am Soc Hematol 125(21):3223–3229

28. Atef B, Azmy E, Aladle D, Mabed M (2019) The prevalence and prognostic significance of autoimmune cytopenias in a cohort of Egyptian patients with chronic lymphocytic leukemia. Hematol Oncol Stem Cell Ther 12(2):97–104

29. Mueller L, Abunafeesa H, Fernandez DC, Kuriakose P (2018) Primary ver-sus secondary autoimmune hemolytic anemia: a retrospective analysis of diagnostic patterns and treatment outcomes. Blood 132:4881

30. Tantiworawit A, Eiamprapai P, Hantrakool S, Chai-adisaksopha C, Rat-taritammrong E, Norasetthada L (2014) Clinical characteristics and long term outcomes of warm type autoimmune hemolytic anemia. Blood 124(21):4874

31. Fattizzo B, Michel M, Giannotta JA, Hansen DL, Arguello M, Sutto E et al (2021) Evans syndrome in adults: an observational multicenter study. Blood Adv 5(24):5468–5478

32. Barcellini W, Fattizzo B (2021) How I treat warm autoimmune hemolytic anemia. Blood 137(10):1283–1294

33. Calvaruso V, Craxì A (2013) Immunological alterations in hepatitis C virus infection. World J Gastroenterol: WJG 19(47):8916

34. No C (2004) Hepatitis associated autoimmune haemolytic anaemia. Int J Pathol 2(1):44–46

35. Sykia A, Gigi E, Sinakos E, Bibashi E, Bellou A, Raptopoulou-Gigi M (2009) Severe autoimmune hemolytic anemia complicated with liver decom-pensation and invasive aspergillosis in a patient with chronic hepatitis C during treatment with peg-interferon-a and ribavirin. J Gastrointest Liver Dis: JGLD 18(1):118–119

36. Chiao EY, Engels EA, Kramer JR, Pietz K, Henderson L, Giordano TP et al (2009) Risk of immune thrombocytopenic purpura and autoimmune hemolytic anemia among 120 908 US veterans with hepatitis C virus infection. Arch Intern Med 169(4):357–363

37. Mohammed RHA, ElMakhzangy HI, Gamal A, Mekky F, El Kassas M, Mohammed N et al (2010) Prevalence of rheumatologic manifestations of chronic hepatitis C virus infection among Egyptians. Clin Rheumatol 29(12):1373–1380

38. Zaki N, El-Naggar A, Ghallab O, Farrag NA, Ebrahim MA (2017) Anemia in Egyptian chronic hepatitis C patients: study of the causes prior to and during interferon/ribavirin versus sofosbuvir therapy. World J Pharmaceut Med Res 3:20–28

39. Ramos-Casals M, García-Carrasco M, López-Medrano F, Trejo O, Forns X, López-Guillermo A et al (2003) Severe autoimmune cytopenias in treatment-naive hepatitis C virus infection: clinical description of 35 cases. Medicine 82(2):87–96

40. Abdallah GE, Abbas WA, Elbeih EA, Abdelmenam E, Saleh MFM (2021) Systemic corticosteroids in the treatment of warm autoimmune hemo-lytic anemia: a clinical setting perspective. Blood Cells Mol Dis 92:102621

Publisher's Note