Original Research Article

Autoimmune Haemolytic Anaemia in Patients with Cancer Diagnoses

ABSTRACT

Aims: To determine if AIHA plays a role in anaemia associated with malignancies, and ascertain the cancers in which AIHA occurs.
Study design: This was a cross-sectional case-control study
Place and Duration of Study: Department of Haematology and Blood Transfusion, University of Port Harcourt Teaching Hospital, Rivers, Nigeria.
Methodology: We conducted the study on patients with malignancies either on admission in the wards or attending follow up clinics. Healthy age/sex matched subjects were used as controls. Cases with and without chemotherapy were analyzed as sub groups. Three hundred and seventy six (376) participants (188 cancer patients and 188 controls) were enrolled in the study. Full blood count, reticulocyte count, blood film, direct antiglobulin test (DAT), indirect antiglobulin test (IAT) and bilirubin assays were conducted on anticoagulated blood samples of all the patients and controls. The DAT was performed on fresh sample not more than 6 hours after collection using polyspecific anti-human globulin.
Results: Three (1.6%) of the 188 patients with malignancies were found to have a positive DAT of which 2 (1.1%) had strongly positive DAT with features of haemolysis and therefore had AIHA. The two patients with AIHA had chronic lymphocytic leukaemia. The third case was a weak positive DAT with malignant teratoma but did not have features of haemolysis. The cases of both AIHA and DAT were found in the group without chemotherapy. AIHA was the aetiology of anaemia in 2 (2%) of the 98 cases who had anaemia and were chemotherapy naive.
Conclusion: AIHA plays a minor role in the aetiology of anaemia in cancer and is more common in lymphoid malignancies. A positive DAT may occur without features of haemolysis.

Keywords: Autoimmune haemolytic anaemia, AIHA, cancer, haemolytic anaemia, anaemia

1. INTRODUCTION

Autoimmune haemolytic anaemia (AIHA) is a heterogeneous group of diseases characterized by autoantibody production to red cell antigens causing shortened survival of the red cells. Anaemia caused by red blood cell (RBC) autoantibodies is not common.[1] In 1946 it was observed that the red cells of patients with acquired haemolytic anaemia sometimes had a positive direct antiglobulin test (DAT) but patients with hereditary haemolytic anaemias were DAT negative and this led to the discovery of AIHA due to the development of autoantibody to the red cells.[2]
AIHA has an incidence of 1–3 cases per 100,000/year.[1] More than half the cases of AIHA are warm AIHA with an incidence of 1/50,000 - 80,000 persons.[3,4] About 1 in 1,000 - 36,000 healthy blood donors may have a positive DAT result.[5] AIHA can be broadly grouped into two: primary (or Idiopathic) and secondary, based on the presence or absence of an underlying disease. Idiopathic AIHA is more common in females with a peak incidence in the fourth and fifth decades of life.[1] Up to 40% cases of AIHA are associated with an underlying disorder especially in lymphoid

malignancies.[6] The red cell antigens important for binding of autoantibodies include the Rh antigen system and the I,i antigen system. Depending on the optimal temperature of reactivity of the autoantibody, AIHA may be warm (usually IgG at 37^0C), cold (usually IgM at 0-4^0C), biphasic (due to a cold reacting IgG- Donath-Landsteiner antibody) or mixed AIHA with features of both warm and cold type. The type of antibody attached determines the rate or site of haemolysis, the propensity to fix complement and the clinical features of the disease.[7]
Serologically detectable warm-reactive autoantibodies do not always result in haemolysis. Majority of patients with AIHA have an associated primary clinical condition and the strength of the DAT reactivity for IgG and C3 correlates with the presence or absence of haemolysis.[5]

Autoimmune phenomena have been reported in both haematological and solid malignancies.[8] Autoimmunity and malignancy frequently coexist and may share aetiological and pathological mechanisms. In both conditions there is associated impaired cellular regulation. In autoimmune diseases autoantibodies and autoreactive lymphocytes are due to immune dysregulation while in malignancies there is impaired regulation of cellular maturation and proliferation which causes uncontrolled neoplastic growth. Immune dysregulation is believed to play a pathogenic role in the development of both autoimmunity and neoplasia. Normally there is immune surveillance with response against cancer cells. This is because the cancer cells produce tumour associated antigens (TAAs) which express HLA class I molecules and so are recognized by T-cells as "foreign" which leads to death of the cancer cells. Production of "autoantibodies" to these TAAs causes death of cancer cells.[8, 9] There is an increased incidence of autoantibodies in patients with neoplastic diseases. Conversely, an increased incidence of malignancies occurs in autoimmune diseases. AIHA is the most common autoimmune disorder associated with malignancies.[10] The exact pathogenesis of the association between carcinoma and AIHA is not fully understood. Cancer immune dysregulation may lead to loss of tolerance and subsequent development of AIHA.[2,11] The AIHA secondary to neoplasia may be due to; release of TAA by the tumour which mimic red cell membrane antigens, production of autoantibodies by the tumour itself (in B-cell lymphomas) or secondary adsorption of immune complexes formed from immune reaction to the tumour on the RBC membrane.[8, 12]
Similarities in the pathogenesis of autoimmunity and cancer include; elderly age, genetic predisposition, aberrant apoptosis, the use of immunosuppressive agents in the treatment of one disorder, which may lead to the development of the other and chronic infections (chronic antigenic stimulation of lymphocytes may lead to susceptibility of malignant transformation or autoimmunity).[8,12,13, 14]

Autoimmune haemolytic anaemia has been associated with mostly lymphoid malignancies like chronic lymphocytic leukaemia (CLL) than non-lymphoid or solid malignancies, however it has also been reported in solid tumors including renal cell carcinoma, Kaposi sarcoma, breast, gastric cancers, of the lungs, breasts, ovaries, colon, caecum, cervix, hypernephroma and melanoma.[15,16,17,18,19] Up to a third of CLL patients have a positive DAT with 5-25% cases of CLL are associated with AIHA, usually by 4.1 years after the diagnosis of CLL was made.[3, 20] The incidence of AIHA in non-Hodgkin's lymphoma (NHL) is reported to be 13.7%.[21] AIHA associated with malignancy usually subsides on successful treatment of the tumour[22] and autopsies of patients with AIHA associated with solid tumours demonstrated autoreactive IgG within the tumour location.[23]

Paradoxically, lymphoproliferative neoplasms or other cancers may arise as a consequence of autoimmunity, including AIHA.[13,24] For example, rheumatoid arthritis predisposes to the development of lymphoma. In Sjogren's Syndrome, there is a 44 times increased risk of developing lymphoma. The presence of AIHA increases the risk of NHL by 2.6 fold.[25] Out of 175 patients on follow up for AIHA, a malignancy was found in 14% of them.[8] About 18% of AIHA cases subsequently develop a clinically diagnosed lymphoproliferative neoplasm within an average of two years.[21,14] In a large series of 1,463 patients with warm AIHA to evaluate the relationship between AIHA and cancer, 31% had a malignancy (CLL occurred most frequently, followed by NHL, solid tumours were seen in 5%.[26]

The diagnosis of AIHA is based on the presence of anaemia, a positive direct antiglobulin test (DAT) with signs of haemolysis (reticulocytosis, low haptoglobin, increased lactate dehydrogenase, elevated unconjugated bilirubin). Sometimes, not all of these typical features are present.[23] Although AIHA is uncommon, it should be considered in the differential diagnosis of anaemias associated with cancers, especially if the patient has a concomitant lymphoproliferative disorder.[1]
The laboratory diagnosis of AIHA is made when there is a positive DAT in the presence of anaemia and features of haemolysis which include reticulocytosis, increased lactate dehydrogenase, hyperbilirubinaemia, reduced haptoglobin and sometimes haemosiderinuria.[26] The DAT has a sensitivity of about 95%,[27] with false negative and false positive rates in about 2-4% and 8% of all cases respectively.[7]

2. MATERIAL AND METHODS

The aim of this study is to determine the prevalence of AIHA in cancer patients in Port Harcourt, if AIHA plays a role in the pathogenesis of anaemia seen in patients with cancer diagnoses and to assess which cancers are associated with AIHA.

This cross-sectional case-control study was conducted on cancer patients, using healthy age-sex matched subjects as controls. Subjects recruited into this study were patients with any morphologically/ histologically diagnosed cancer who gave written informed consent to participate in the study. In the case of participants less than 18 years, consent was obtained from the parent or legal guardian. Those who had received blood transfusion in the preceding three months were excluded from the study. The control group consisted of healthy age-sex matched subject without cancer diagnoses or any chronic disease. Pregnant women were excluded. Subjects who fulfilled these criteria were selected in a systematic manner. Approval for this research was given by the institution's ethics committee.

Sample Size determination:

The sample size expression;

$$n = \frac{л\,(1-л)}{e^2}$$

to estimate a quantity of interest with a specified precision was used to determine the sample size,[28] where:

e= usually set at 0.005

л= the estimated prevalence of the particular characteristic in the target population. In this case using the prevalence of AIHA in CLL with a prevalence rate of 7%;[29]

$$n = \frac{0.07\,(1-0.07)}{0.005} = 13$$

Using the above estimate, a minimum sample size of 13 for each of the common haematological and non-haematological cancers was used to carry out the study. A total of 376 subjects were enrolled in this study (188 cases and 188 controls).

Venous blood was collected by venipuncture from all participants and analyzed for haemoglobin concentration, peripheral blood film, reticulocyte count, direct antiglobulin test, indirect antiglobulin test and bilirubin assays (total & unconjugated). All tests were carried out in duplicates.

The haemoglobin (Hb) concentration and packed cell volume (PCV) were done using an auto-analyzer. Hb was regarded as low if 11g/dl or less in males, 10g/dl or less for adult females and 10g/dl or less in children below 18 years. Peripheral blood film was made using Leishman stain to assess for the presence of polychromasia, spherocytes or fragmented cells.

Reticulocyte count was done manually using new methylene blue stain and the number of reticulocytes per 1,000 red cells was counted. The corrected reticulocyte count was calculated using the formula below, taking the expected PCV for females and children as 36% and that for males as 40%. The normal reticulocyte count was taken as 0.5 – 1.5%.

$$\textit{Corrected Reticulocyte Count} = \frac{\textit{Reticulocyte count (\%)} \times \textit{Patient's PCV (\%)}}{\textit{Average normal PCV (\%)}}$$

A direct antiglobulin test was done to determine the presence of autoantibodies on the red cells of subjects, using polyspecific anti-human globulin which detects both IgG and complement fragment C3. An indirect antiglobulin test (IAT) was done on the samples with a positive DAT to determine if the autoantibody is 'pan-reactive'. The IAT detects antibodies that are present in the serum of subjects.

Total and unconjugated bilirubin assays were done by the department of chemical pathology based on the Jendrassik and Groff method. Normal values for total bilirubin and unconjugated bilirubin were taken as 5-17 μmol/L and <8.5μmol/L respectively.

Data was analyzed using statistical software package- Microsoft Excel®.

3. RESULTS AND DISCUSSION

A total of 376 participants (188 patients with cancer diagnoses and 188 age-sex matched controls), consisting of 69 adult males, 98 adult females and 21 children <18 years. The mean age of the total population was 46.5 ±20 years (range- 5months to 89 years). Of the total cases, 149 (79.3%) were chemotherapy naïve, while 39 (20.7%) were on chemotherapy. The demographics of the cases is stated in table 1.

The haemoglobin concentration (Hb Conc.) for the total cases ranged from 3.3 – 15.3g/dl (mean 9.6g/dL ± 2.2). The prevalence of anaemia in the total cancer population was 64.9% (122 cases: 47 adult males, 62 adult females and 13 children). There was no statistically significant difference in the prevalence of anaemia between the chemotherapy naïve

cases (65.1%, mean Hb conc. 8.1g/dL, range 6.3 – 9.7g/dL) and the cases on chemotherapy (64.1%, mean Hb conc. 8.3g/dL, range 3.3 – 10.7g/dL). In this study it appears chemotherapy does not make subjects to be more anaemic (p=0.53). Only 5 (2.7%) of the control population had anaemia, p= <0.00001. A summary of the variables are listed in tables 2 and 3.

Although 33 of the total 122 cases (27%) with anaemia had reticulocytosis, only 6 (4.9%) had a true reticulocytosis using the mean reticulocyte count corrected to 40% for adult males and 36% for adult females and children (corrected reticulocyte count). Of the total cancer population with anaemia, reticulocytopenia was seen in 29 cases (23.8%) using the absolute reticulocyte count. Only one participant from the control group had reticulocytosis with a corrected reticulocyte count of 2.7%.

Table 1: Distribution of cases based on exposure to chemotherapy

	Chemotherapy Naïve Cases		Cases on Chemotherapy		Total CASE Population	
	Number (%)	Mean Age (Years)	Number (%)	Mean Age (Years)	Number (%)	Mean Age (Years)
Adult Males	60 (40.3%)	55.1 ± *19.9*	9 (23.1%)	46.7 ± *20*	69 (36.7%)	54 ± *16.4*
Adult Females	74 (49.7%)	49.1 ± *18.8*	24 (61.5%)	52.0 ± *14.1*	98 (52.1%)	49.8 ± *13.5*
Children	15 (10%)	6.4 ± *3.2*	6 (15.4%)	5.3 ± *1.9*	21 (11.2%)	6.1 ± *4*
TOTAL	**149 (79.3%)**	**47.2 ± 19.6**	**39 (20.7%)**	**43.6 ± 22**	**188 (100%)**	**46.5 ± 20.1**

Table 2: Summary of variables based on exposure to chemotherapy among Cases

	CHEMOTHERAPY NAÏVE CASES				CASES ON CHEMOTHERAPY				TOTAL CASES
	Adult Males	Adult Females	Children <18 years	TOTAL	Adult Males	Adult Females	Children <18 years	TOTAL	
(Number)	60	74	15	**149**	9	24	6	**39**	**188**
$\bar{X}$ Age (years)	55.1±15.7	49.1±13.3	6.4±4.6	**47.2±19.6**	46.7±20	52.0±14.1	5.3±1.9	**43.6±22**	**46.5±20.1**
$\bar{X}$ Hb conc. *(g/dL)*	9.8±2.4	9.5±2	9.2±2.3	**9.6±2.3**	8.6±1.4	9.6±2.3	9.6±2	**9.4±2.1**	**9.6±2.2**
Freq. of Anaemia	39 (65%)	47 (63.5%)	11 (73.3%)	**97 (65.1%)**	8 (88.9%)	15 (62.5%)	2 (33.3%)	**25 (64.1%**	**122 (64.9%)**
$\bar{X}$ Retic. Count (%)	1.6±1.4	1.5±1.5	2±2.6	**1.6±1.6**	2.8±2.1	1.4±1.1	0.78±0.6	**1.6±1.5**	**1.6±1.6**
$\bar{X}$ Cor. Retic (%)	0.96±0.6	1±0.8	0.99±0.6	**1±0.7**	1.6±1.3	1±0.7	0.69±0.6	**1.1±0.9**	**1±0.7**
Freq. of Polychrom.	13 (21.7%)	15 (20.3%)	5 (33.3%)	**33 (22.1%)**	5 (55.6%)	7 (29.2%)	1 (16.7%)	**13 (33.3%)**	**46 (24.5%)**

Freq. of Spherocyte	6(10%)	4(5.4%)	1(6.7%)	**11(7.4%)**	1(11.1%)	1(4.2%)	0(0%)	**2(5.1%)**	**13(6.9%)**
Freq. of Schistocyte	7(11.7%)	1(1.4%)	1(6.7%)	**9(6%)**	2(22.2%)	1(4.2%)	1(16.7%)	**4(10.3%)**	**13(6.9%)**
$\bar{X}$ T. Bil (µmol/L)	10.6±6	12.9±14	9±3.1	**11.6±10.6**	9.9±5	9.9±3.1	7.9±2.6	**9.6±3.6**	**11.2±9.6**
$\bar{X}$ Unconj. Bil (µmol/L)	6.7±3.5	7.1±4.2	6±1.4	**6.8±3.7**	7.4±2.2	5.8±1.6	5.75±1.9	**6.2±1.9**	**6.7±3.4**
Freq. of DAT +ve	2(3.3%)	1(1.4%)	0(0%)	**3(2%)**	0(0%)	0(0%)	0(0%)	**0(0%)**	**3(1.6%)**
Freq. of AIHA	1(1.7%)	1(1.4%)	0(0%)	**2(1.3%)**	0(0%)	0(0%)	0(0%)	**0(0%)**	**2(1.06%)**

$\bar{X}$-Mean; *Hb conc*- Haemoglobin concentration; *Freq*- Frequency; *Retic.*- Reticulocyte; *Cor. Retic*- Corrected Reticulocyte; *Polychrom.*- Polychromasia; *T.Bil*- Total bilirubin; *Unconj. Bil*- Unconjugated bilirubin; *DAT*- Direct Antiglobulin Test; *AIHA*- Autoimmune haemolytic anaemia

Table 3: Comparison of variables between cases based on chemotherapy and between the total cases & controls

Variable	No Chemo (149)	Chemo (39)	*p*-value	CASES (188)	CONTROLS (188)	*p*-value
$\bar{X}$ Hb (g/dL)	9.61	9.37	0.53	9.56	12.78	<0.0001*
$\bar{X}$ Retic (%)	1.61	1.61	0.99	1.61	1.08	<0.00001*
$\bar{X}$ Corr. Retic (%)	1.00	1.08	1.00	1.02	1.11	0.17
Freq. of Polychromasia *(N, %)*	33 (22.1%)	13 (33.3%)	0.15	46 (24.5%)	12 (6.5%)	<0.00001*
Freq. of Spherocytosis *(N, %)*	11 (7.4%)	2 (5.1%)	0.62	13 (6.9%)	3 (1.6%)	0.01*
Freq. of Schistocytes *(N, %)*	9 (6%)	4 (10.3%)	0.36	13 (6.9%)	4 (2.2%)	0.03*
$\bar{X}$ TBil (µmol/L)	11.60	9.63	0.06	11.19	9.48	0.03*
$\bar{X}$ UBil (µmol/L)	6.85	6.16	0.11	6.71	6.28	0.12
Freq. of DAT+ *(N, %)*	3 (2%)	0 (0%)	0.37	3 (1.6%)	0 (0%)	0.08
Freq. of AIHA *(N, %)*	2 (1.3%)	0 (0%)	0.47	2 (1.1%)	0 (0%)	0.15

*statistically significant p-value <0.05

$\bar{X}$-Mean; *Hb conc*- Haemoglobin concentration; *Freq*- Frequency; *Retic.*- Reticulocyte; *Cor. Retic*- Corrected Reticulocyte; *T.Bil*- Total bilirubin; *Unconj. Bil*- Unconjugated bilirubin; *DAT*- Direct Antiglobulin Test; *AIHA*- Autoimmune haemolytic anaemia

The cancers in this study were both haematological (n=65, 34.6%) and non-haematological cancers (n=122, 64.9%) including a peculiar case of mixed cancer in a female that had both breast cancer and chronic myeloid leukaemia (CML)-

see table 4. The direct antiglobulin test (DAT) was positive in 3 patients in the total cancer population, 2 (both adult females) of whom had chronic lymphocytic leukaemia (CLL) and the third was a case of malignant teratoma in an adult male (table 5). Of the total 188 cases, only 2 (1.1%) had strongly positive DAT (4+, 3+) with anaemia and features of haemolysis, and therefore had AIHA. The case of malignant teratoma had a weak DAT (1+), mild anaemia no features of haemolysis and was taken as a case of positive DAT only. These 3 cases that were DAT positive also had agglutination on the indirect antiglobulin test (IAT).

The 2 of the 14 cases of CLL who had AIHA in the total cancer population (14.3%) belonged to the chemotherapy naïve group, which had 12 of the total 14 cases of CLL giving a prevalence of 16.7% of AIHA in that arm. AIHA was found in 4.2% of the 48 patients with lymphoproliferative neoplasm (LPN). There were 34 mature lymphoid neoplasms (chronic lymphocytic leukaemia, non-Hodgkins lymphoma, Hodgkin's lymphoma, multiple myeloma) and AIHA was found in 5.88% of them. Autoimmune haemolytic anaemia was present in 2 (1.3%) of the 149 cases in the chemotherapy naïve group and 2 (1.06%) of the total 188 cases with cancer. AIHA accounted for 2 of the 97 chemotherapy naïve cases with anaemia (2.1%) and 2 of the 122 cases with anaemia in the total cancer population (1.6%). There was no case of AIHA in the cancer group on chemotherapy, but this was not statistically significant (p= 0.47). The control group did not record any positive DAT nor AIHA, although this difference was also not statistically significant. (*p*=0.08 & 0.15 respectively).

Table 4: Breakdown of Types of Cancers in participants and DAT Prevalence

<table>
<tr><th colspan="9">TYPES OF CANCERS</th></tr>
<tr><td colspan="3">Haematological Cancers</td><td colspan="3">Non-Haematological Cancers</td><td colspan="3">Mixed Cancers</td></tr>
<tr><td colspan="3">(65 Cases)</td><td colspan="3">(122 Cases)</td><td colspan="3">(1 Case)</td></tr>
<tr><td>Type</td><td>No.</td><td>DAT +</td><td>Type</td><td>No.</td><td>DAT +</td><td>Type</td><td>No.</td><td>DAT +</td></tr>
<tr><td colspan="3">Myeloid Neoplasm</td><td>Bladder</td><td>14</td><td>0</td><td rowspan="11">Breast & CML</td><td rowspan="11">1</td><td rowspan="11">0</td></tr>
<tr><td>CML (14)</td><td rowspan="2">17</td><td rowspan="2">0 (0%)</td><td>Breast</td><td>17</td><td>0</td></tr>
<tr><td>AML (3)</td><td>Prostate</td><td>15</td><td>0</td></tr>
<tr><td colspan="3">Lymphoid Neoplasm</td><td>Cervix</td><td>14</td><td>0</td></tr>
<tr><td>ALL (13)</td><td>13</td><td>0 (0%)</td><td>Colon</td><td>14</td><td>0</td></tr>
<tr><td>CLL (14)</td><td>14</td><td>2 (14.3%)</td><td>Ovarian</td><td>14</td><td>0</td></tr>
<tr><td>NHL (13)</td><td rowspan="3">21</td><td rowspan="3">0 (0%)</td><td rowspan="3">OTHERS*</td><td rowspan="3">34</td><td rowspan="3">1 (2.9%)</td></tr>
<tr><td>HL (3)</td></tr>
<tr><td>MM (5)</td></tr>
</table>

CML- Chronic myeloid leukaemia; AML- Acute Myeloid Leukaemia; ALL- Acute Lymphoid Leukaemia; CLL- Chronic Lymphoid Leukaemia; NHL- Non-Hodgkin's Lymphoma; HL- Hodgkin's Lymphoma; MM- Multiple Myeloma;

(OTHERS included: nephroblastoma, primary liver cell carcinoma, parotid cancer, rhabdomyosarcoma, choriocarcinoma, gastric cancer, Kaposi sarcoma, melanoma, malignant teratoma, Dermatofibrosarcoma, colorectal cancer, oesophageal cancer, vulval cancer, layryngeal cancer, Liposarcoma, lung cancer & Chondrosarcoma.)*

TABLE 5: Characteristics of the three cases with positive DAT

	Cancer	Hb	Retic	C.Retic	Pol.	Sph	T-Bil	U-Bil	DAT	IAT	AIHA
1	CLL	8	3.4%	2.8%	++	++	25	20	++++	+++	Yes
2	CLL	8.6	5.5%	3.9%	+++	+	34	21	+++	++	Yes
3	Malignant Teratoma	9.7	1.9%	1.4%	-	-	11	5.6	+	+	No

*Hb conc.- Haemoglobin concentration (g/dl); Retic-Reticulocyte count; C.Retic-Corrected Reticulocyte count; Pol- Polychromasia; Sph- Spherocytosis; T-Bil- Total Bilirubin (µmol/L); U-bil- Unconjugated bilirubin (µmol/L)

It is estimated that about 50% -64% of cancer patients will be anaemic.[30,31,32] The survey in this study shows that of the 188 total cancer cases, 122 (64.9%) had anaemia. Although chemotherapy is a known cause of anaemia, it did not appear to affect the prevalence of anemia in the chemotherapy group, compared to the chemotherapy naïve group (64.1% vs 65.1%, p=0.54). There was a low mean Hb concentration of the total cancer population irrespective of chemotherapy. The control group had a normal mean Hb concentration for age and sex, although 4.7% of the control group had anaemia.

A useful indicator of bone marrow erythropoietic activity is the reticulocyte count and the corrected reticulocyte count determines how efficient erythropoiesis is. The proportion of patients with reticulocytosis were more in the group receiving chemotherapy (19.2% compared to 8.7% in those without chemotherapy), whereas the patients without chemotherapy had more cases of reticulocytopenia (16.4%) compared to 11.5% in the chemotherapy group. From the total cancer population, about a third of the anaemic population had reticulocytosis (35.4%) with the uncorrected reticulocyte count, but this was further reduced to only about a tenth of them having reticulocytosis using the corrected reticulocyte count. Therefore, the anaemia in this survey was mostly hypoproliferative anaemia.

In this study, a positive DAT was seen in 3 cases with cancer, and none in the control group. The DAT positive cancer cases were all not on chemotherapy. The 3 DAT positive cases were made up of 2 cases of CLL (both females above the age of 60 years) and 1 malignant teratoma (male, 33 years). Autoimmune phenomena are more common in females and increases with age as was seen in our study.[33] The DAT positive CLL patients had anaemia with features of haemolysis (AIHA) but the case of malignant teratoma with a PCV of 29% had no features of haemolysis. AIHA occurs most frequently in lymphoid neoplasms and CLL is the most common LPN associated with AIHA.[3] No positive DAT was seen in the group receiving chemotherapy; this may be due to the effect of chemotherapy on AIHA.[23]

AIHA is the commonest autoimmune phenomena in cancer[13] and occurs more in haematological malignancies (especially lymphoid malignancies)[34] as was seen in this study. There is an association between CLL and AIHA in our study. The diagnosis of AIHA is usually made at about 4.1yrs after diagnosis of CLL.[3] Our study is a cross-sectional study and therefore we cannot say how long after diagnosis of CLL it took to develop AIHA. Although AIHA may also precede the onset of CLL,[26] this too cannot be estimated from this cross-sectional study.

There was no record of a positive DAT or AIHA in the myeloid neoplasms. Although AIHA has been reported in several solid malignancies, our study did not record any case of AIHA in the non-haematological malignancies. However, there was a case of positive DAT in the solid malignancies but without haemolysis. A positive DAT may be found in 7-8% of hospitalized patients.[35] The DAT positive anaemia in this study that did not have reticulocytosis or features of haemolysis may fall into this category, however reticulocytopenia may also occur in AIHA.[36] Therefore although this study indicates that 1 of the 3 DAT positive patients did not have features of AIHA, it is possible that the patient still had AIHA due to apoptotic phenomenon that could occur in some cases of patients with AIHA reticulocytopenia may be a feature rather than reticulocytosis. The case of DAT positive anaemia may be at risk of developing AIHA or a lymphoid malignancy in the future.[5] The cases with AIHA in this study had anisocytosis due to polychromasia and spherocytosis on their blood films. The case of positive DAT without haemolysis had a relatively normal blood film.

The autoantibodies in AIHA result in panagglutination thereby causing confusion during the crossmatch process. The three cases with positive DAT in this study had incompatible crossmatch with pooled blood group O red cells, using the indirect antiglobulin test. In this study only a small fraction (1.1%) of the subjects with cancer had AIHA. Therefore although AIHA may cause anaemia; it only plays a minor role in the aetiology of anaemia in cancers.

4. CONCLUSION

Anaemia is common in patients with cancer although the mechanisms by which it occurs are diverse. Autoimmune haemolytic anaemia does play a role in the anaemia of cancer even though it accounts for a very small percentage. AIHA occurred more frequently in patients with chronic lymphocytic leukaemia. In addition, this data shows that it is possible to have a positive direct antiglobulin test in the absence of features of haemolysis (therefore a positive DAT does not always equate to AIHA). The prevalence of 1.6% for positive DAT and 1.1% for AIHA in cancer patients from our study is higher than in the general population (0.01%).[1,5] We have also documented the presence of AIHA in cancer patients, but only in haematological malignancies. We suggest a larger study to confirm these observations. We recommend that although AIHA is uncommon, patients that have anaemia in cancer especially with features of haemolysis should also have a DAT done as part of their investigations.

LIST OF ABBREVIATIONS

AIHA	Autoimmune haemolytic anaemia
CLL	Chronic lymphocytic leukaemia
CML	Chronic myeloid leukaemia
DAT	Direct antiglobulin test
Hb	Haemoglobin
Hb conc.	Haemoglobin concentration
IAT	Indirect antiglobulin test
LPN	Lymphoproliferative neoplasm
NHL	Non-Hodgkin's lymphoma
PCV	Packed cell volume
RBC	Red blood cell
TAA	Tumour associated antigens

COMPETING INTERESTS

The Authors declare that we have no competing interests.

CONSENT & ETHICAL APPROVAL

Informed consent was obtained from all participants in the study. Ethical approval was obtained from the hospital Ethics Board where the study was conducted.

REFERENCES

[1] Go, RS., Winters JL, Kay NE. "How I treat autoimmune hemolytic anemia." Blood 129.22 (2017): 2971-2979.

[2] Dacie JV.; Autoimmune Haemolytic Anaemias; British Medical Journal, 1970, 2,381- 386

[3] Petz LD, Garraty G; Classification and characteristics of autoimmune hemolytic anemias; Chapter 3 in Petz LD, Garraty G; Immune Hemolytic Anemias, 2nd edition
2004: 61-131

[4] Domen RE. An overview of immune hemolytic anemias. Cleve Clin J Med. 1998;65:89-99

[5] Rottenberg Y,Yahalom V,Shinar E, et al. Blood donors with positive direct antiglobulin tests are at increased risk for cancer. Transfusion 2009; 49: 838–842.

[6] Dean L; Blood Groups and Red Cell Antigens: (Internet): Bethesda; 2005; Chapter 2, Blood Group antigens are surface markers on the red blood cell membrane; [Cited 22 July 2015]
http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=rbcantigen&part=ch2

[7] Kalfa TA. Warm antibody autoimmune hemolytic anemia. Hematology Am Soc Hematol Educ Program. 2016; (1):690-697.

[8] Achenza MIS, Selmi C. Autoimmunity and Cancer. Asian Pacific Journal of Cancer Prevention; Vol 13, 2012, 29-40

[9] Liu W, Peng B, Lu Y, Xu W, Qian W, Zhang JY; Autoantibodies to tumour associated antigens as biomarkers in cancer immunodiagnosis. Autoimmune Reviews 2011; 10 (6): 331-335

[10] Tomer Y, Shoenfeld Y: Cancer and Autoimmunity, 2000, Chapter 15, Pages 141-150

[11] Jayapal V: Fundamentals of Medical Immunology; Jaypee 2007: 196-208, 254-275, 417-441

[12] Tomer Y, Shoenfeld Y; Autoantibodies, Autoimmunity and Cancer; in Shoenfeld Y, Gershwin ME; The Decade of Autoimmunity. Elsevier Science B.V. 2000, 141-150

[13] Sallah S., Wan J.Y, Hanrahan L.R; Future Development of Lymphoproliferative Disorders in Patients with Autoimmune Hemolytic Anemia; Clinical Cancer
Research, 2001, Vol. 7, 791–794

[14] Stern M, Buser AS, Lohri A, Tichelli A, Nissen-Druey C; Autoimmunity and malignancy in hematology- More than an association. Critical Review in Oncology Hematology 2007; 63 : 100-110

[15] Puthenparambil J, Lechner K, Kornek G. Autoimmune hemolytic anemia as a paraneoplastic phenomenon in solid tumors: A critical analysis of 52 cases reported in the literature. Wien Klin Wochenschr. 2010 Apr;122 (7-8):229-36. doi: 10.1007/s00508-010-1319-z.

[16] Ugoeke N, Onweni C, Treece J, et al. Inflammatory Breast Cancer and Warm Antibody Autoimmune Hemolytic Anemia: A Rare Paraneoplastic Syndrome. J Investig Med High Impact Case Rep. 2017;5(4):2324709617740905.

[17] Morris PG, Swords R, Sukor S et al; Autoimmune Hemolytic Anemia Associated With Ovarian Cancer: Journal of Clinical Oncology; Sept 2008, 17,4993-4994

[18] Agrawal K, Alfonso F. A Rare Association of Autoimmune Hemolytic Anemia with Gastric Adenocarcinoma. Case Reports in Oncological Medicine, Vol. 2017, Article ID 8414602, 5 pages, 2017

[19] Algaze SD, Park W, Harrington TJ, Mudad R. Autoimmune haemolytic anaemia in a patient with advanced lung adenocarcinoma and chronic lymphocytic leukaemia receiving nivolumab and intravenous immunoglobulin Case Reports 2018; 2018:bcr-2017-221801.

[20] Mittal S, Blaylock MG, Culligan DJ, Barker RN, Vickers MA; A high rate of CLL phenotype lymphocytes in autoimmune hemolytic anemia and immune thrombocytopenic purpura. Haematologica Jan 2008, 93 (1) 151-152

[21] Köksal Y, Çaliskan Ü, Uçar C, Erekul S, Ilerisoy-Yakut Z.; Autoimmune hemolytic anemia as presenting manifestation of primary splenic anaplastic large cell lymphoma. Turk J Pediatr 2006; 48: 354-356

[22] Valent P, Lechner K; Diagnosis and treatment of autoimmune haemolytic anaemias in adults: a clinical review. Wiener Klinische Wochenschrift, 2008;120(5-6):136-51

[23] Cooper R, Tappin J.A; Bronchial carcinoma presenting as autoimmune haemolytic anaemia: Postgraduate Medical Journal, 1981 57, 528-529

[24] Hoffman PC; Immune Hemolytic Anemia—Selected Topics: Hematology, Jan 2006; 2006: 13 – 18

[25] Fozza C, Longinotti M; Use of Rituximab in autoimmune hemolytic anemia associated with non-Hodgkin's lymphoma. Advances in Hematology 2011; 2011 Article ID 960137: 1-4

[26] Reardon JE, Marques MB; Laboratory Evaluation and Transfusion Support of Patients With Autoimmune Hemolytic Anemia: Am J Clin Pathol 2006;125(Suppl 1):S71-S77

[27] Michel M; Characteristics of warm autoimmune hemolytic anemia and Evans syndrome in adults. Presse Medicale. 2008 Sep;37(9):1309-18

[28] Kirkwood BR, Sterne JAC; Calculation of required sample size; Chapter 35 in Essential Medical Statistics. Blackwell Publishing, 2nd Edition 2003; 413-428

[29] Visco C, Novella E, Peotta E, Paolini R, Giaretta I, Rodeghiero F; Autoimmune hemolytic anemia in patients with CLL is associated with IgVH status. Haematologica 2010; 95 (7): 1230-1232

[30] Mercadante S, Gebbia V, Marrazzo A, Filosto S; Anaemia in cancer: pathophysiology and treatment. Cancer Treat Rev. 2000 Aug;26(4):303-11

[31] Varlotto J, Stevenson MA; Anaemia, tumour hypoxaemia and the cancer patient. Int. J. Radiation Oncology Biol. Phys, 2005; 63 (1) 25-36

[32] Moliterno AR, Spivak JL; Anaemia of cancer; Hematology Oncology Clinic North America 1996; 10 (2) 345-63

[33] Fairweather D, Rose NR; Women and autoimmune diseases. Emerging Infectious Diseases 2004; 10 (11) 2005-11

[34] Teachey D, Felix C: Development of Cold Agglutinin Autoimmune Hemolytic Anemia During Treatment for Pediatric Acute Lymphoblastic Leukemia Journal of Pediatric Hematology/Oncology. 2005:27 - Issue 7:397-399

[35] Petz LD, Garraty G; The serologic investigation of autoimmune hemolytic anemia: Chapter 6 in Petz LD, Garraty G; Immune Hemolytic Anemias 2nd Edition 2004; 201-230

[36] Van de Loosdrecht AA, Hendriks DW, Blom NR, et al. Excessive apoptosis of bone marrow erythroblasts in a patient with autoimmune haemolytic anaemia with reticulocytopenia. Br J Haematol. 2000;108:313-315