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Clinicopathological Study of Non-Immune Thrombocytopenia in Adults

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Abstract: ***Background:Thrombocytopenia is the most common cause of abnormal bleeding. The causes of thrombocytopenia are diverse but they can be grouped according to the pathogenesis into immune and non-immune mechanisms. This prospective study focuses on the analysis of adult patients confirmed with thrombocytopenia using clinical and relevant laboratory parameters to arrive at a possible aetiopathogenesis. Aims and Objectives: To evaluate patients with Thrombocytopenia presenting to the Clinical Pathology, Medical and Haematology OP departments of Government General Hospital and Madras Medical College and establish the pathogenetic mechanisms. Materials and Methods: This prospective study was conducted in the Goschen Institute of Pathology, Madras Medical College from 2008-2009. Patients presenting to the Clinical Pathology and Medical and Hematology OPD who were found to have thrombocytopenia, defined as a platelet count less than 150 x 10^9 / Lt., in an automated counter and confirmed by peripheral smear examination were included in the study. Data including detailed History, Clinical examination and some mandatory laboratory test were done for all patients . Additional tests were done when required. .Patients who after detailed clinical assessment and laboratory tests were diagnosed to have an immunological basis for thrombocytopenia were excluded from the study***

Keywords: Thrombocytopenia, Peripheral blood smear, Bone Marrow aspirate smear, Coagulation Profile.

1. Introduction

Thrombocytopenia is the most common cause of abnormal bleeding. Based on etiopathogenesis, the causes can be immunological, non-immunological, Splenic sequestration or Pseudo-Thrombocytopenia. Though the differential diagnosis of Thrombocytopenia is broad, the cause can be recognized easily in some cases. But, often, especially in ominous cases, it requires a detailed workup, including extensive history, Clinical examination and Laboratory Investigations. This study analyses the adult patients confirmed with Thrombocytopenia using Clinical and relevant Lab parameters to arrive at a possible etiopathogenesis.

2. Literature Survey: Approach to a patient with Thrombocytopenia

2.1 Definition

Thrombocytopenia is defined as a platelet count below 150 x 10 ^{9}/L . Thrombocytopenia results from mainly four processes-artifactual thrombocytopenia, deficient platelet production accelerated platelet destruction and abnormal distribution or pooling of platelets within the body. Artifactual thrombocytopenia or falsely low platelet counts occurs exvivo when platelets are not counted accurately which may occur in the presence of giant platelets or with platelet satellitism.[1], [2]. The most common cause of artifactual thrombocytopenia is platelet clumping. The presence of platelet clumps on examination of the peripheral blood smear and normal platelet count using citrated blood confirms pseudo thrombocytopenia as the cause. Electronic cell counters sometimes show false low counts due to giant platelets. If repeated samples show low counts which do not correlate with the clinical parameters, it is mandatory to confirm platelet count by manual counting methods and to confirm the count by a well stained peripheral smear. If thrombocytopenia is confirmed, a stepwise evaluation should be undertaken to assess the cause [3]-as given in the algorithm

2.2 Problem definition

Thrombocytopenia is a common condition encountered in clinical practice. The unravelling of the etiopathogenetic mechanism is of utmost importance in the management of the patients with Thrombocytopenia. A comprehensive history, clinical examination and salient relevant laboratory tests can provide this in most cases. If improperly managed, the consequences can some times be vey disastrous due to bleeding at various sites. A prompt assessment of the pathogenesis helps the clinician to manage the patients better.

2.3 Methodology

This prospective study was conducted in the Goschen Institute of Pathology, Madras Medical College from 2008-2009. The study included 34 subjects who presented to the Clinical Pathology,Haematology and Medical OP departments of Madras Medical College.

Inclusion Criteria: Patients presenting to the clinical pathology and medical OP departments who were found to have thrombocytopenia, defined as a platelet count less than 150 x 10^9 / L in an automated counter and confirmed by peripheral smear examination.

Exclusion Criteria: Patients who, after detailed clinical assessment and laboratory tests were diagnosed to have an immunological basis for thrombocytopenia were excluded from the study.

A detailed history was elicited from each patient included in the study population including Chief presenting complaints and duration, the history of present illness, Past history (of chemotherapy / radiotherapy, Malignancies, previous surgery or blood transfusion), drugs ingested in the past (with emphasis on intake of indigenous medicines), previous bleeding tendencies and site of bleeding ,chronic ailments (like diabetes mellitus, systemic hypertension, bronchial asthma, ischaemic heart disease, pulmonary tuberculosis, epilepsy), Personal history (of alcohol intake-quantity, duration, Tobacco / paan intake and duration, Extra marital sexual contact), Menstrual history (in women - age at menarche, duration of the menstrual flow, regularity and duration of the menstrual cycle, age at menopause)and Obstetric history (in women- marital status, number of children, abortions).

General examination: Included assessment of vital parameters, nutritional status, pallor, icterus, clubbing, pedal oedema, evidence of bleeding, sternal tenderness, lymphadenopathy. Other features were assessed if warranted on the basis of provisional diagnosis

Systemic examination: Assessment included examination of cardiovascular, respiratory, central nervous system and abdomen.A provisional clinical diagnosis was made with differential diagnosis and necessary laboratory tests were performed.

Laboratory tests: Peripheral venous blood obtained from antecubital venipuncture was used. Appropriate amounts of blood were transferred into tripotassium EDTA vacutainer for complete blood count, sodium citrate 3.2 % for coagulation profile and plain blood in sterile tubes for biochemical analysis. Some blood was preserved at -20^{o}C for special tests. The **complete blood count** was performed using SYSMEX KX-21 3 part cell counter. The following parameters were included: Total leucocyte count, differential count, haemoglobin, hematocrit, MCV, MCH, MCHC, red cell distribution width, platelet count, mean platelet volume and platelet distribution width. ESR was measured by Westergren's method. **Peripheral blood smear** stained by Leishman stain was examined.

Bone marowstudy: Bone marrow aspirate was obtained from posterior superior iliac crest using 16G bone marrow aspirate needle. Smears were stained with Leishman stain. Bone marrow iron studies were performed in some cases. In relevant cases bone marrow trephine biopsy was performed and H & E stained paraffin sections were examined.

Coagulation profile: Blood with appropriate amount of 3.2 % sodium citrate was used for prothrombin time, activated partial thromboplastin time and fibrinogen estimation. PT, aPTT and fibrinogen was done using coagulatory method in SYSMEX CA-500. D-dimer levels were estimated in select cases.

Biochemical tests: Plain blood was used for assessing liver function, renal function and estimation of serum lactate dehydrogenase.

Ultrasonogram was performed in all cases.

For patients with suspected infections, the following tests were done:Smears for MP, MF and QBC method, MSAT for Leptospirosis, DengueIgG and IgM antibodies, HIV- I & II antibodies by rapid and ELISA method, Blood culture for enteric and non-entric organisms, Gram Stain, Stain for AFB, fungus, Sputum examination , Rheumatoid factor, ANA and ACLA

When malignancy was suspected, CT scan, MRI, Fluid analysis for effusions including cytological study, FNAC in cases of palpaple masses. eg., cervical lymphadenopathy and Histopathology to exclude or confirm malignancies were done.

3. Results and Discussion

All the cases were analysed and categorized according to the final diagnosis. The distribution was as follows. (Table 1).

Table 1: Distribution of Cases

S.No	Category	No. of Cases
1.	Decreased Production	15
2.	Ineffective haematopoeisis	3
3.	Hypersplenism with congestive splenomegaly	1
4.	Infections	7
5.	Connective Tissue Disease	4
6.	Drug Induced	2
7.	Disseminated Intravascular Coagulation	1
8.	Microangiopathichaemolyticanaemia	1

4. Haematological Malignancies

Thrombocytopenia is well known in acute haematological malignancies due to extensive infiltration of the marrow by blasts replacing normal haematopoietic elements.[4] .Our study also showed a significant reduction in megakaryocytes in the marrow in all the cases of acute leukaemia. Our study included 6 cases of AML,3 cases of ALL(Figs 1,2,3,4**).** One case of AML M5 in addition showed dysplastic megakaryocytes in the marrow and a significant prolongation of PT and APTT.The dysplasia producing an ineffective megakaryopoiesis and increased coagulation parameters with gastrointestinal bleeding suggestive of an underlying disseminated intra vascular coagulation could be additional contributory factors to the thrombocytopenia. Further this patient was on the 4th cycle of chemotherapy with a possible drug induced myelosuppression. Another case of AML presented as pancytopenia with prolonged APTT. The possibilityof myelodysplastic syndrome converting to AML was considered[5] . The patient had a history of native medicine intake the same probably contributing to the myelodysplasia and hypoplastic kidney probably chronic pyelonephritis. One case of ALL had a significant elevation of LDH with a prolongation of PT and APTT. He was partially treated outside.The possibility of tumourlysis syndrome with DIC was considered. One patient presented with pancytopenia[6] with bone marrow showing lymphoblasts and FNAC of cervical node suggesting a lymphoproliferative disorder. Two cases of CML presented with thrombocytopenia.One patient had epistaxis, arthralgia and fever, a leucoerythroblastic blood picture, 18% myeloblasts,bone marrow showing 42% myeloblasts, dysplasia and occasional hemo phagocytosis. Increased

reticulo endothelial cells,prolonged PT, reduced fibrinogen and increased LDH were also observed. Apart from the conversion of CML to AML accounting for the thrombocytopenia, the possibilities of myelodysplastic syndrome with ineffective megakaryopoiesis and haemophagocytic histiocytosis as evidenced by increased reticulo endothelial cells and haemophagocytosis in the marrow, increased prothrombin time, decreased fibrinogen and increased LDH were considered. The second case of CML presented with pancytopenia. She was on treatment with Hydroxyurea and Busulphan based on the availability. Marrow showed extensive fibrosis with a few clusters of megakaryocytes. The following possibilities were considered-drug induced fibrosis and evolving megakaryoblastic leukemia

Hypoplastic Disorders: We had 4 cases of aplastic anaemia in our study.One patient had an absent left kidney and a scaly skin with mild dystrophy of toe nails suggestive of possible dyskeratosiscongenita.

Ineffective Haematopoiesis: Although a common disorder, our study included only one case of megaloblastic anaemia in a young female who responded well to B12 and folate, ineffective megakaryopoiesis being the main contributor to thrombocytopenia. There were two cases of primary MDS in this study, both patients being symptomatic for the pancytopenia including thrombocytopenia. Marrow showed trilineal dysplasia, ineffective haematopoiesis contributing to thrombocytopenia. In addition dysplasia was noted in 2 denovo acute leukaemia and CML in blast crisis. Dysplasia(secondary) was also noted in two cases of connective tissue disorder,one drug induced thrombocytopenia and a patient with HIV.

Our study had only one case of hypersplenism with splenic sequestration ie a case of decompensated liver disease with portal hypertension and congestive splenomegaly. Hepatotropic viral markers were negative and patient also had GradeIII oesophageal varices, decreased fibrinogen ie 112 mg/dL and ascites. Hepatic coagulopathy was considered as an additional factor to thrombocytopenia.

Infections

Four cases of acute febrile illness with thrombocytopenia were included in the study. 2 were positive for Dengue IgG, IgM. Peripheral destruction of platelets with cross reacting antibodies is the common pathogenetic mechanism for thrombocytopenia[6].One patient had in addition an increased LDH with marrow showing haemophagocytosis with a possibility of sequestration of platelets in the macrophages as an additional contribution to thrombocytopenia. One patient had decreased megakaryocytes in the marrow and reactive lymphocytes in smear (Fig.5).The third case of acute febrile illness presented with a prolonged pancytopenia with the marrow appearing cellular with partial maturation arrest in granulocytic series, a few megakaryocytic bare nuclei and haemophagocytosis. With the APTT prolongation, clinical features of bleeding and evidence of haemophagocytosis the possibility of virus associated macrophage activation was considered[7],[8] The fourth case also presented with an acute febrile illness and was MSAT positive for Leptospirosis. In addition to immune mediated platelet destruction, inhibited platelet production was considered in the pathogenesis of thrombocytopenia. Two cases of malariaP.vivax infestation.(Fig.6) presented with thrombocytopenia,the pathogenesis of thrombocytopenia being probably due to [9],[10] splenic sequestration and enhanced macrophage activity and activation of platelets by haemolysed red cells resulting in DIC.APTT was prolonged in one patient**.** Quinine and sometimes chloroquine are sometimes known to cause thrombocytopenia. There was no evidence of renal dysfunction in both these cases. We had one patient with HIV presenting with thrombocytopenia. Marrow appeared hypocellular with dysplastic changes. The patient also had Aspergillus pneumonia (Fig.7,8).The following contributors to thrombocytopenia were considered-HIV induced marrow changes**,**Drug induced myelopathy and Myelodysplasia with ineffective megakaryopoiesis**.** There was no evidence of sepsis or DIC in this patient.

Connective Tissue Disease

4 cases of connective tissue disease were included in this study because of possible non immune mechanisms contributing to thrombocytopenia. One case of SLE presenting with pancytopenia, bleeding diathesis, prolonged APTT, low fibrinogen, increased LDH, the marrow showing adequate haematopoietic elements, increased reticuloendothelial cells with haemophagocytosis. She had evidence of E.coli infection at the time of admission. The possibility of macrophage activation syndrome was considered with a good response to appropriate antibiotics and plasmapheresis**.** A case of Juvenile rheumatoid arthritis also presented with features of macrophage activation syndrome with severe pancytopenia and hepatic dysfunction as evidenced by grossly increased serum alkaline phosphatase and coagulation parameters, low fibrinogen and increased LDH, clinical hepatospenomegaly and generalized lymphadenopathy. We had a third interesting case of a 46/F who presented with thrombocytopenia and thrombosis.ANA and ACLA were positive. Hams test was negative. She was on steroids and Acitrom. In 6 months, she presented with an abdominal wall hematoma with a grossly prolonged APTT and persistent thrombocytopenia. The peripheral blood showed circulating normoblasts and occasional red cell fragments. The increased APTT with a fairly normal PT and deep seated intramuscular bleeding suggested possibility of acquired antibodies to coagulation factors. The thrombocytopenia, apart from the immune mediated platelet destruction, could be aggravated due to dysplasia in megakaryocyte series**.** One case of ankylosing spondylitis, a young male presented with anaemia and thrombocytopenia with a prolonged APTT. He presented with mild splenomegaly and was on NSAID for spondyloarthropathy. Marrow appeared hypercellular with increased megakaryocytes with micromegakaryocytes, moderate myeloid hyperplasia, increased reticuloendothelial cell activity. The following possibilities were considered-Increased disease activity as evidenced by increased reticuloendothelial cells, increased ESR, prolonged APTT and low normal fibrinogen and macrophage engulfment of platelets and NSAID induced peripheral destruction due to

cross reacting antibodies and myelopathy resulting in dysplasia/suppressions.

Drug Induced Thrombocytopenia

NSAID induced thrombocytopenia included one case of ankylosing spondylitis. A case of tuberculosis on class I Anti tuberculous therapy for 2months which included Rifampicin developed thrombocytopenia. Marrow evaluation showed decreased megakaryocytes and no granuloma or fibrosis. The patient was subsequently put on a regime without Rifampicin and platelet counts gradually recovered[11],[12], [13].

5. Disseminated Intravascular Coagulation

A 52 year old male on treatment for non small cell lung carcinoma with a positive cytology in pleural and ascitic fluid and endobronchial biopsy developed a bleeding diathesis while on therapy. The complete blood count showed pancytopenia, increased coagulation parameters low normal fibrinogen, increased fibrin degradation products and D-dimer, increased LDH. The marrow aspirate was hypocellular and the trephine biopsy showed necrotizing granulomatous inflammation negative for AFB(The patient was however on ATT before the diagnosis of malignancy).The cause of thrombocytopenia in this patient could be attributed to Marrow infiltration by necrotizing granulomata ? Tuberculosis? Carcinomatosis and increased coagulation parameters and LDH with a d-dimer increase – Disseminated carcinoma with a chronic consumption.

Red Cell Fragmentation

A 28 year old male with a prosthetic cardiac valve for Rheumatic heart disease for over 20 years presented with fever, breathlessness and bleeding gums. Laboratory data showed a severe thrombocytopenia, low normal fibrinogen and increased LDH. The marrow showed myeloid hyperplasia with adequate megakaryocytes with peripheral smear showing mild anisocytosis with occasional fragmented RBC.USG showed minimal splenomegaly. The possibility of Chronic red cell fragmentation with underlying subclinical platelet consumption compounded by a subsequent infection (infective endocarditis) resulting in severe thrombocytopenia and clinical bleeding.

The analysis of 34 cases of thrombocytopenia revealed an interesting mix of etiopathogenetic factors. It was possible to deduce to a reasonable level the pathogenetic mechanisms which in many cases were confirmed by response to treatment on follow up of patients.

6. Summary and Conclusion

Thrombocytopenia with or without bleeding manifestations is a common problem in general clinical practice. To treat or not to treat and how to treat would depend a lot on etiopathogenesis of this problem. As pathologists, it is for us to highlight this point in order to help the clinician manage the patient in a scientific and beneficial manner. As we have seen, supportive parameters like a good peripheral smear examination, coagulation profile, comprehensive marrow evaluation and certain biochemical parameters with the clinical profile go a long way in narrowing the etiology and maximizing treatment benefits. It should be remembered that all thrombocytopenias are not immune mediated. Also in cases of confirmed immune thrombocytopenia one should have an open mind about other non immune processes creeping into pathogenesis during progression of disease.

7. Future Scope

This is a limited study and evaluation of larger number of patients is therefore necessary for a more comprehensive and comparative analysis.

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Author Profile

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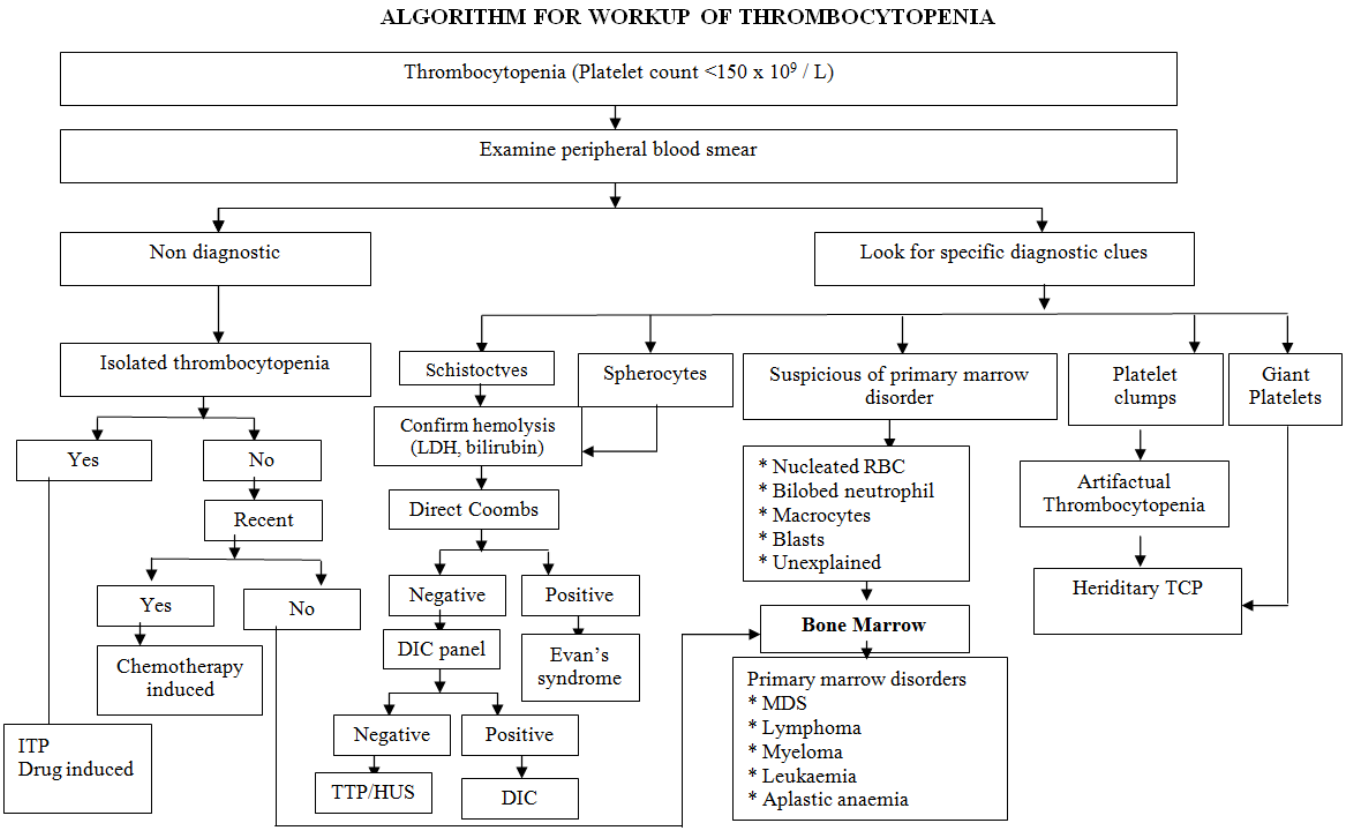

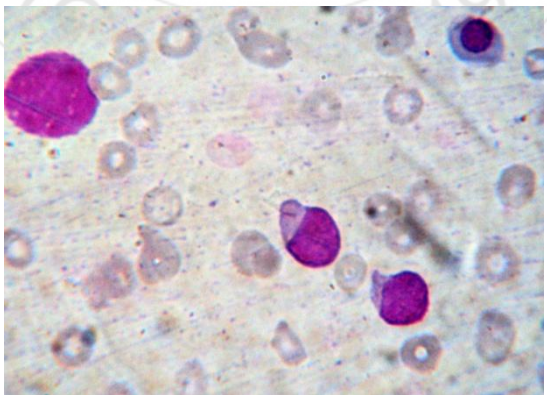

Figure 1: AML M2 Myeloblast showing Auer rod. Leishman x1000

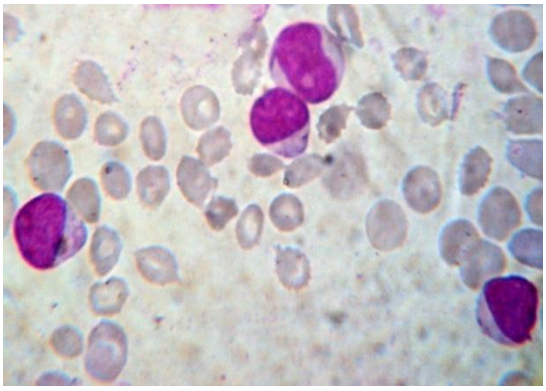

Figure 2: Myeloblast showing Prominent nucleoli rod. Leishman x1000

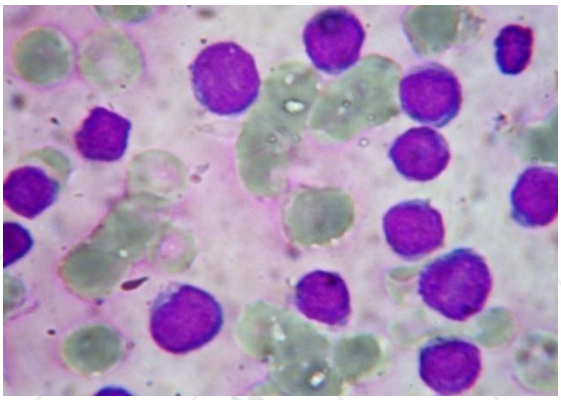

Figure 3: ALL showing lymphoblasts. Leishman x1000

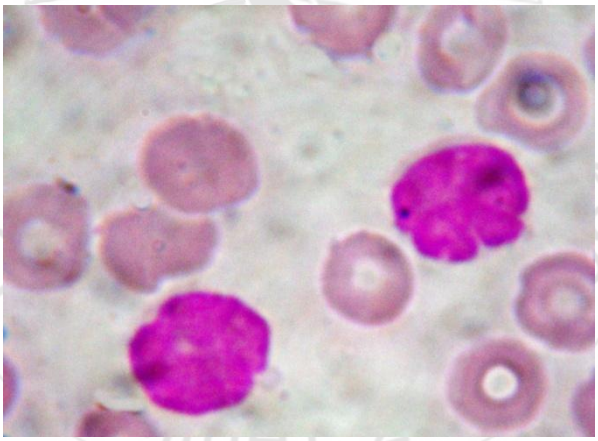

Figure 4: ALL showing Lymphoblasts with cleaved nuclei leishman x 1000

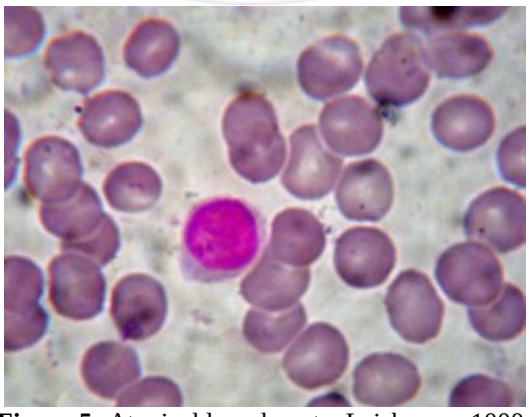

Figure 5: Atypical lymphocyte. Leishman x1000

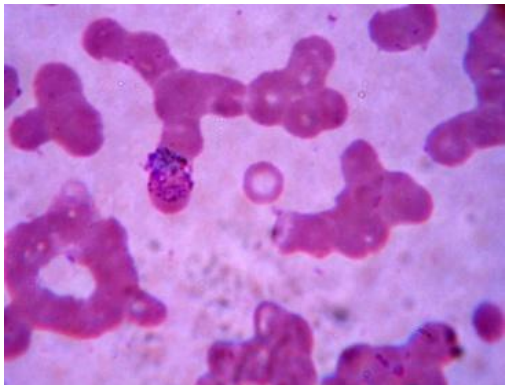

Figure 6: Trophozoites of P. vivax with thrombocytopenia Leishman x1000

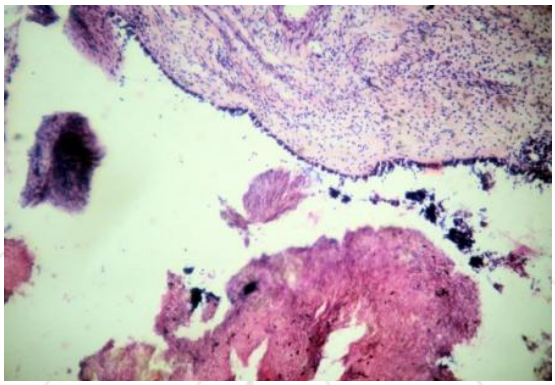

Figure 7: Endobronchial Biopsy showing hyphae of Aspergillus. H & E x100

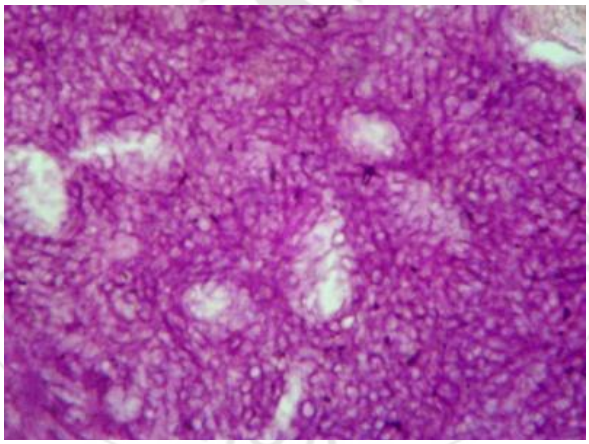

Figure 8: Hyphae of Aspergillus. H & E x400