IMMUNE-RELATED PANCYTOPENIA : A RARE CASE REPORT IN SURABAYA, INDONESIA

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ABSTRACT : Immune related pancytopenia (IRP) is a peripheral pancytopenia caused by bone marrow failure due to an autoimmune process. A 41 year old female presented with gum bleeding 4 days prior to admission. She had anemic conjunctiva and no organomegaly. Laboratory results were as follows Hb: 7.9 g/dL, WBC: 3,200/ìL, PLT: 6,000/ìL, IPF: 4.7% and RET: 0.44%. Bone marrow aspiration was dominated by clusters of erythroblastic islands and activated macrophages. Confirmatory examination with bone marrow mononuclear cells coombs test (BMMNC Coombs Test) showed positive results. The patient received dexamethasone and trasnfusions with both packed red cells (PRC) and platelet concentrate (PC) apheresis. Mechanism of pancytopenia in patients is an autoimmune process caused by an autoantibody towards hematopoeitic cells in the bone marrow. B lymphocytes over produces this autoantibody. Ig G autoantibody also activates macrophages that will phagocyte hematopoeitic cells, whereas the Ig M autoantibody activates the complement system that causes the lysis of hematopoietic cells. The diagnosis of IRP in this patient was based on peripheral pancytopenia with an increase of Erythroblastic Island clusters and activated macrophages in the bone marrow. The BMMNC Coombs Test confirmed the diagnosis.

Key words : IRP, Erythroblastic Islands, BMMNC Coombs test.

INTRODUCTION

In early 1998, there were patients presenting with pancytopenia that did not suit the diagnostic criteria for either hematological nor non-hematological diseases. Some patients responded well towards corticosteroids and high dose intravenous immunoglobulin therapy (He *et al*, 2001). The cause of pancytopenia in some patients were due to an autoimmune process that was caused by an autoantibody towards the membrane of hematopoietic cells of the bone marrow. autoantibodies were detected on the membrane of bone marrow hematopoietic cells by the bone marrow mononuclear cells (BMMNC) Coombs test, a modification of the peripheral blood coombs test, using BMMNC Coombs test to detect the binding of autoantibodies to bone marrow hematopoietic cells (BMHCs) (Bing *et al*, 2012; Xiao *et al*, 2020). At first, some of these patients were diagnosed as atypical aplastic anemia (AA), early stage AA, proliferative AA or chronic AA. In the 1980's, some patients were diagnosed as myelodysplastic syndrome (MDS) due to a bad response towards AA therapy. Due to the indication of the involvement of the body's immune system in the disease pathogenesis, these abnormalities were then called immune related pancytopenia (IRP), marked by cytopenia and a positive BMMNC Coombs test (Fu *et al*, 2014).

IRP cases are rarely reported and there is still limited data in China. Wang *et al* (2014) reported 81 cases of IRP in China and Liu *et al* (2012) also reported 20 cases in China. Patients characteristics were cytopenia or pancytopenia, normal or high reticulocyte count (RET), hypoplastic bone marrow or hyperplastic bone marrow with an increase of erythroblastic islands, and erythroid hyperplasia (Chasis, 2006; Manwani and Bieker, 2008;

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Lue and Shao, 2012).

Case

Patients Identity : Female, 41 years old

Chief complaint : Weak

History of current disease : Patient presented with weakness for 4 days before arrival, dizziness, no fever, no nausea and vomiting, decrease of appetite, and normal micturition and defecation.

History of illness : Patient was admitted in the hospital a month ago due to the same complaints. Patient got 4 Packed Red Cell (PRC) and 4 Platelet Concentrate (PC) by apheresis transfusions.

Physical examination

General condition : Compos mentis, GCS 456

Vital signs : HR: 101/min; temp: 35.2 C, RR: 24×/min, Blood pressure: 130/90 mmHg.

Head/neck : Anemic conjunctiva.

Thorax : Heart and lungs within normal limit.

Abdomen : Soft, bowel sounds (+), liver and spleen unpalpable.

Extremity : Warm, Capillary refill test <2 sec, no edema, no petechiae.

Supporting Examinations

Table 1 : Patients complete blood count.

Parameter	16/01/20	19/01/20	Normal values
WBC (10³/mL)	3.2	1.96	4.5-13.5
% Neu (%)	42.1	61.7	39.8-70.5
% Eos (%)	0.0	0.0	0.6-5.4
% Baso (%)	0.0	0.0	0.3-1.4
% Mo	28.8	8.2	4.3-10
% Lym (%)	29.1	30.1	23.1-49.9
RBC (10⁶/uL)	2.91	3.45	3.4-5.0
Hb (g/dL)	7.9	9.5	12.0-15.0
Hct (%)	23.3	30.5	35-49
MCV (fL)	80.1	88.4	80-94
MCH (pg)	27.1	27.5	26-32
MCHC (g/dL)	33.9	31.1	32-36
RDW (%)	17.1	17.2	11.5-14.5
Plt (10³/mL)	6	40	150-450
IPF (%)		4.7	1.1-6.1
RET# (10⁶/mL)		0.0152	0.034-0.1
RET %		0.44%	0.8-2.21

Blood Smear Evaluation (16/01/2020)

Erythrocytes : Normochromic normocytic anisocytosis (microcytes), polychromasia cells (+), normoblasts (-).

Leukocytes : Appear decreased normal in number, dominated by segmental neutrophils, atypical lymphocytes (-), immature granulocytes (-), blasts (-).

Thrombocytes : Appear decreased in number, giant platelets (-)

Impression : Normochromic normocytic anisocytosis anemia, leukopenia, thrombocytopenia

Conclusion : Peripheral pancytopenia with inadequate bone marrow response due to the low IPF and RET.
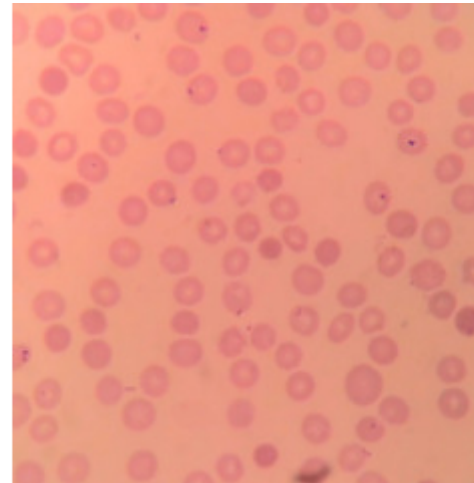

Fig. 1 : Patients' blood smear evaluation showing pancytopenia.

Bone Marrow Aspiration (17/01/20)

Location : Right SIPS

Consistency : Normal

Cellularity : Difficult to evaluate due to dilution

ME Ratio : 1:6

Erythropoeitic System : Highly increased activity, 75% proportion, mild dysplasia (+) (intercellular bridging, multinucleated normoblasts).

Granulopoeisis System : Decreased activity, 12% proportion

Megakariopoeisis System : Decreased activity, difficulty in finding megakaryocytes, dysplasia (+)

Other cells : No non-hematopoietic cells present.

Conclusion : Bone marrow is dominated by clusters of erythroblastic islands and activated macrophages, peripheral pancytopenia and this description suggests an IRP.

Advice : BMMNC Coombs test

BMNNC Coombs Test Results (21/01/20)

Direct Coombs Test : Positive 4+

Clinical diagnosis and follow up

Patient was diagnosed by the clinician as pancytopenia and suspected of MDS with a differential
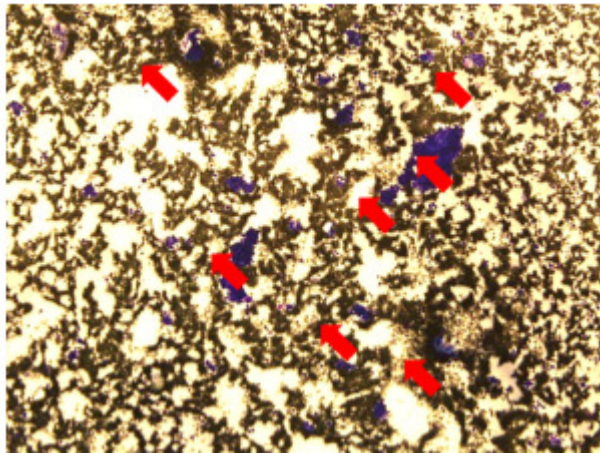
Fig. 2 : Bone Marrow aspiration shows an increase in clusters of erythroblastic islands.
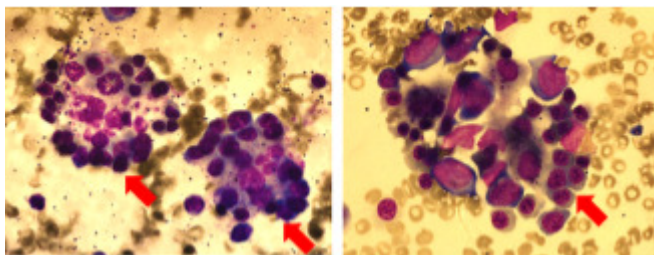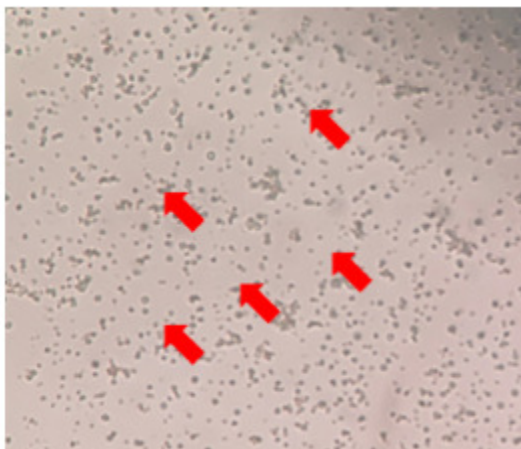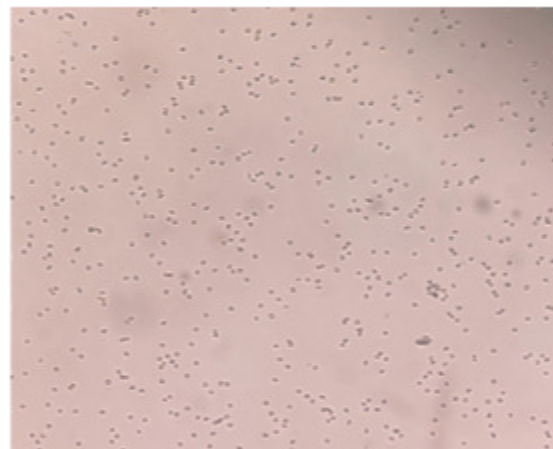

Fig. 3 : Bone Marrow Aspiration shows an increase in clusters of erythroblastic islands and activates macrophages.

Fig. 4 : BMMNC Coombs test using gel method, shows a group of red blood cells agglutinated on the top of the well which is interpreted as positive 4+.

confirmation of the criteria of suspecting IRP; (b) a positive BMMNC Coombs test and/or flow cytometry (FCM) analysis; (c) good response toward adrenocortical and/or high dose intravenous immunoglobulin therapy if the patient has a negative BMMNC Coombs test and/or FCM analysis. The diagnosis of this case is based on the detection of autoantibody in bone marrow cells of BMMNC Coombs test and/or FCM analysis. The exclusion of other hematopoietic diseases is very

Patients' (+) BMMNC Coombs Test   **Control**

Fig. 5 : BMMNC Coombs test using the slide method shows small and big agglutination of red blood cells that are interpreted as positive.

diagnosis of AA and was given dexamethasone, transfusion of PRC and transfusion of PC apheresis.

DISCUSSION

The criteria for suspicion of IRP, based on analysis of clinical data from various sources are: (a) cytopenia or pancytopenia with normal or higher percentage of RET and/or neutrophils, hypoplastic bone marrow or hyperplastic bone marrow with an increase of erythroblastic islands and erythrocyte phagocytosis; and (b) exclusion of all hematopoietic and non-hematopoietic causes of cytopenia. Criteria for proving IRP are: (a)

important, such as AA, paroxysmal PNH, MDS, Autoimmune Hemolytic Anemia (AIHA), Evans syndrome, megaloblastic anemia, myelofibrosis and other non hematopoeitic disease (Xiao *et al*, 2020).

The patients complete blood count shows Hb: 7.9 g/dL, WBC: 3,299/mL, PLT: 6,000/mL, IPF: 4.7% and RET: 0.44%. Pancytopenia with no increase in both IPF and RET values, indicates a hematopoietic disorder in the bone marrow. This case also shows an increase of clusters of erythroblastic islands and activated macrophages that indicate this patient is a suspect of IRP.
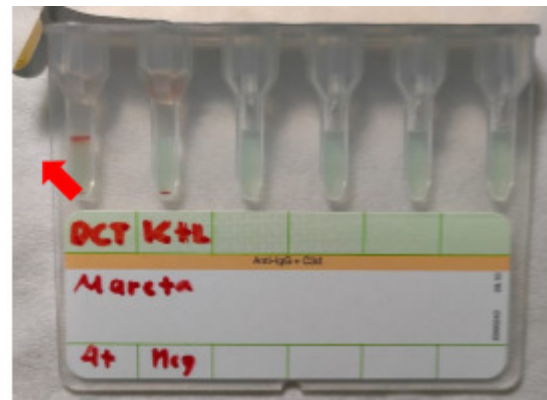
Wang *et al* (2014) stated that patients with IRP will show an increase in erythroblastic island and erythroblasts phagocyted by macrophages in the bone marrow. This is consistent with Wang *et al* (2014) research that states that there are significant differences in Erythroblastic Islands in patients with IRP compared with severe aplastic anemia (SAA) patients and healthy control, which are as follows 7.81±3.78, 0.1±0.31 and 4±1.46 in each bone marrow aspiration with a p <0.0002.

This patient underwent a confirmation test for IRP diagnosis by doing a BMMNC Coombs test with the gel and slide method. The gel method had a result of 4+ and the slide test gave a positive result. These examinations were confirmation tests for patients with IRP. The purpose of these tests was to determine if there was an autoantibody process towards BMMNC. In 2000, Fu *et al* (2003) was the first to detect autoantibody towards BMMNC in an IRP patient using the BMMNC Coombs test. This autoantibody was produced by the B lymphocyte that has an exaggerated function. Ig G autoantibody activates the macrophage to phagocyte hematopoietic cells. The Ig M antibody activates the complement system that causes the lysis of hematopoietic cells (Chen *et al*, 2009). This autoantibody binds to the functional antigen of the membrane, such as erythropoietin receptor. This is in concordance with Liu *et al* (2013) reporting 12 of 20 patients with IRP shows positive BMMNC Coombs test.

IRP is an autoimmune disease that targets the hematopoietic cells of the bone marrow. This is proven by a study on SLE done by Fu *et al* (2003), where more than 50% patients with SLE show a positive BMMNC Coombs test, and 60% have cytopenia. This shows that IRP is an autoimmune disease similar to systemic lupus erythematosus (SLE), but targets the hematopoietic cells of the bone marrow.

The limitations of this case are immunofluorescence test (IF) and FCM haven't been done. Wang *et al* (2014) showed 15 of 20 patients with IRP had positive IgG autoantibody towards hematopoietic cells of the bone marrow, whereas there were no IgG autoantibodies found in patients with AA, MDS and AIHA.

CONCLUSION

In summary, a patient with IRP was reported with cytopenia in the peripheral blood and signs of bone marrow failure. Bone marrow aspiration is dominated with clusters of erythroblastic island clusters and activated macrophage with positive results of BMMNC Coombs Test.

ACKNOWLEDGEMENT

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