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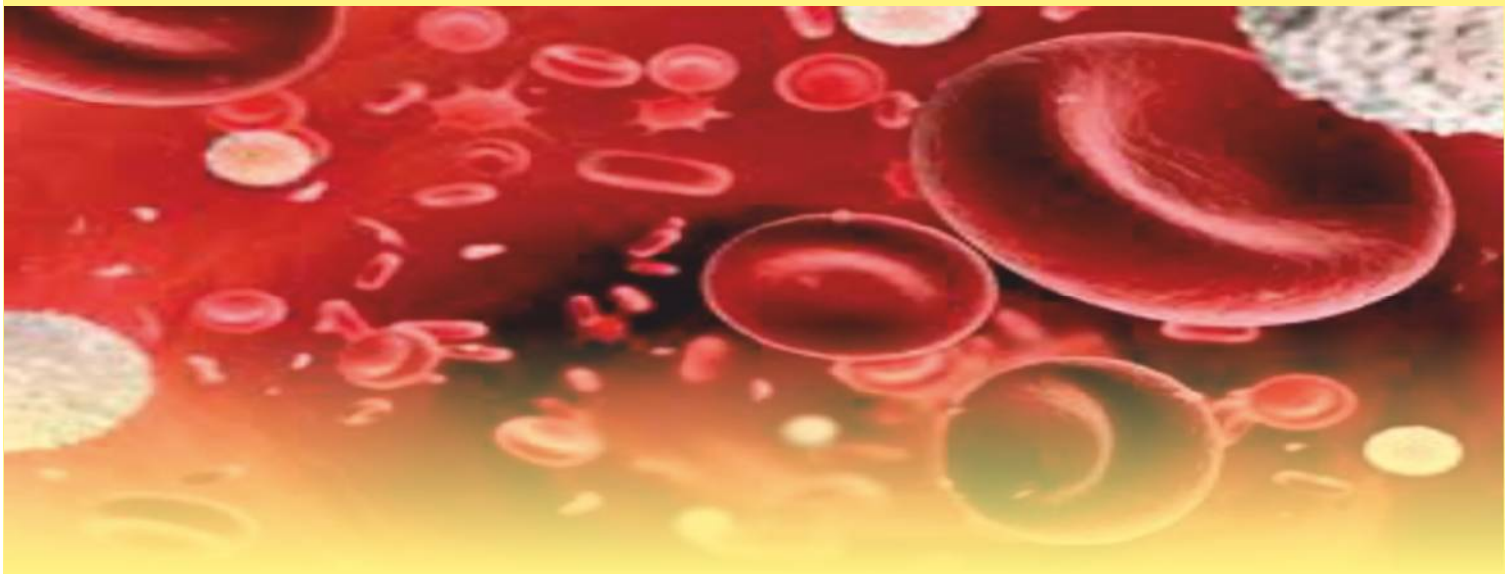

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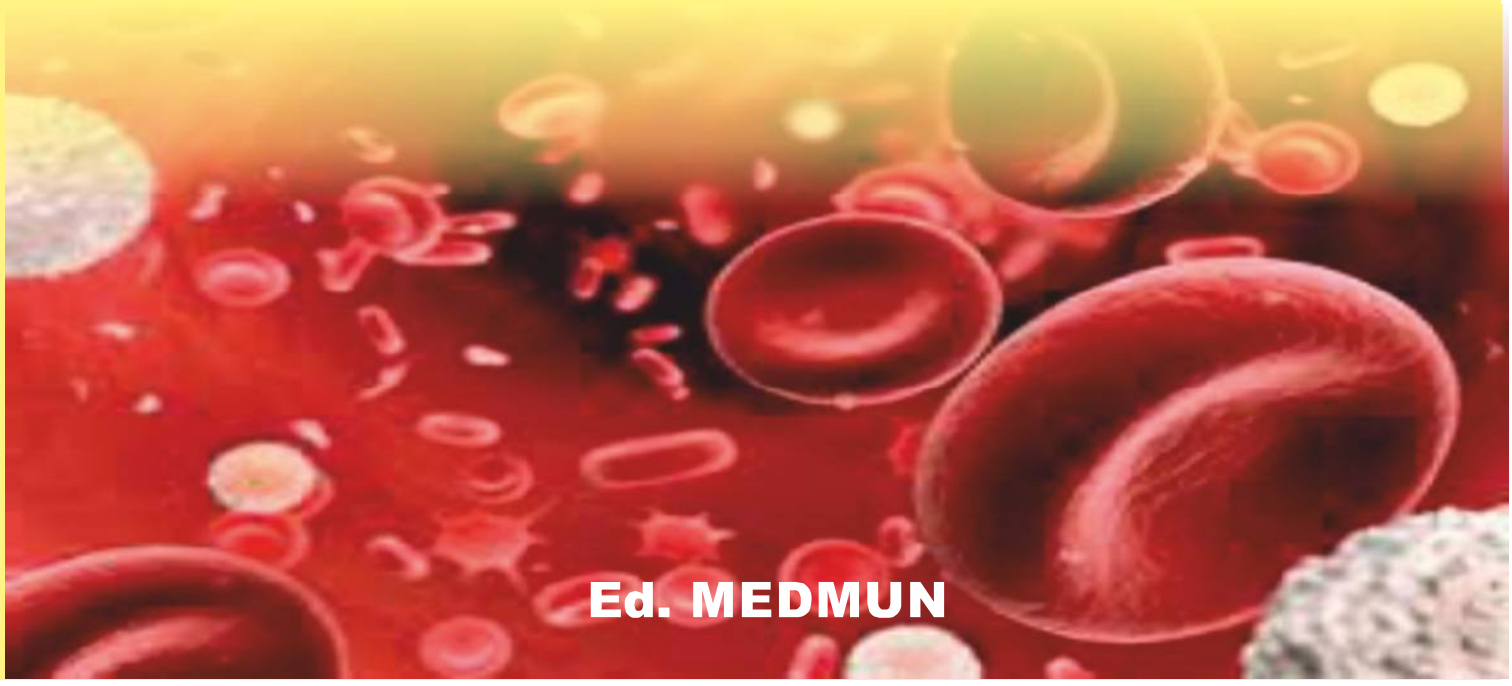

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Updates in Chronic Myeloid Leukemia: Can we do more for our patients?

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Abstract

The management of patients with Chronic Myeloid Leukemia (CML) has changed dramatically with the advent of Imatinib. In our department, the European LeukemiaNet recommendations were adopted for initial evaluation (phases and risk scores), first, second and next line of treatment and evaluation of treatment response. The first tyrosine-kinase inhibitor (TKI) line is tailored according to CML phase and risk score at diagnosis. The response to first TKI line can be initially assessed by cytogenetic analysis of bone marrow metaphases until complete cytogenetic response (CCyR) and/or measurement of BCR-ABL1 transcript levels performed at regular intervals to monitor the depth of response. Patients who fail to respond to first TKI line or lose initial response should be treated with a second TKI line unless they have T315I mutation which would render leukemic cells resistant to all agents except Ponatinib. Allogeneic transplant may be considered for patients with suitable donors as an alternative if they fail second or third line TKI or express T315I mutation.
Key-words: *CML- chronic myeloid leukemia, Ph- Philadelphia chromosome, BCR-ABL1- Breakpoint Cluster Region-Abelson murine leukemia oncogene, TKI-tyrosine- kinase inhibitor.*

Introduction

Chronic myeloid leukemia (CML) is a pluripotent stem cell clone neoplasm, classified as classic form of chronic myeloid neoplasms (MPNs) according to 2008 WHO classification. In more than 90% of patients, CML is characterized by translocation between chromosome 9 and 22 known as Philadelphia (Ph) chromosome. The molecular equivalent of this translocation is Breakpoint Cluster Region- Abelson murine leukemia oncogene (BCR-ABL1) which leads to expansion of erythroid, myeloid and megakaryocytic progenitor populations and reduced response of hematopoietic progenitors to normal regulation stimuli. CML natural evolution is into 3 phases: chronic phase (CP), accelerated phase (AP) and blast crisis or blast phase (BP). Once Imatinib was discovered, prognosis of patients with CML has changed dramatically and has lead to improvement of CML survival. Although Imatinib improves overall survival, not all patients with CML achieve complete cytogenetic and major molecular responses due to primary or secondary resistance. For those patients, the second generation TKI was developed. Allogeneic transplant may be considered for patients with suitable donors as an alternative if they fail second or third line TKI or express T315I mutation which would render their leukemia resistant to all these agents except Ponatinib at any monitoring time.

Diagnosis of CML phases, risk score and treatment options

In our department, the following algorithm[1] (Table 1) was developed for CML diagnosis.

Table 1. Initial work- up for patients with CML

Family and personal history and clinical exam	-vital signs -liver and spleen dimensions (centimeters under costal rebord)	essential
Blood count and differential count	-percents of blasts, basophiles and eosinophiles for risk scores (Sokal, Hasford, EUTOS)	essential
Biochemical tests	-including Ca^{2+}, P^{3-} and LDH	essential
Leukocyte Alkaline Phosphatase	-for differential diagnosis with other type of MPNs and leukemic reaction	not essential
Bone marrow aspirate	-important for blasts percentage	essential
Bone marrow trephine	-for fibrosis grading and blasts percentage	not essential but recommended
Conventional cytogenetic exam	-for evaluation of Philadelphia chromosome and chromosome abnormalities in Philadelphia chromosome positive or negative clone	essential
In situ Hybridization (FISH)	- for cases with no evaluable metaphases at diagnosis, suspicion of cryptic translocations or deletions of chromosome 9 - blood and/or bone marrow samples can be used	not essential; in selected cases
Real time -PCR qRT–PCR	-for qualitative and quantitative evaluation of *BCR-ABL1* transcript, -allows estimation of copies number at any time -important for identification of atypical transcripts	essential

In our department, European LekemiaNet (ELN)[2] criteria for CML are used for chronic (CP), accelerated (AP) or blast (BP) phase definitions. AP and BP criteria are well established but CP includes all patients who did not fulfilled AP and/or BP criteria (Table 2).

Table 2. ELN2013 criteria for accelerated and blast phase

CML phases	Diagnostic criteria
Accelerated phase (AP)	
	-peripheral blood or bone marrow blasts = 15-29% or peripheral blood or bone marrow blasts plus promyelocytes>30% but blasts< 30% - peripheral blood basophiles ≥ 20% - unrelated treatment persistent thrombocytopenia (<100 × 10^9/L) -major chromosomal abnormalities in Philadelphia chromosome positive cells (CCA/Ph+) during treatment
Blast phase (BP)	
	- peripheral blood or bone marrow blasts ≥ 30% -extramedullary blastic proliferation, excluding spleen

For patients with CP of CML, it is mandatory to asses risk since diagnosis to decide best TKI treatment (Table 3).

Table 3.Calculation formulas and risk assessment according to Sokal, Hasford and EUTOS scores

Score	Calculation formula	Risk assessment
Sokal[3]	exp [0,0116 x (age -43,4) + (0,0345/ (spleen dimensions-7,51) + 0,188 x (platelets count/ 700)2-0,563)) + 0,0887 x (peripheral blood blasts-2,1))]	low< 0,8 intermediary= 0,8- 1,2 high> 1,2
Hasford[4]	[0,6666 x age (0 if age < 50 years and1 if age ≥ 50 years) + 0,0420 x spleen dimensions (cm undercostal rebord) + 0,0584 x blasts (%) + 0,0413 x eosinophiles (%) + 0,2039 x basophiles (0 when < 3% and 1 when ≥ 3%) 1,0956 x platelets count (0 if< 1500 x10^9/ L and 1 if ≥ 1500) x 1.000]	low≤ 780 intermediary = 780 ≤ 1480 high> 1480
EUTOS[5]	(7 x basophiles) + (4 x spleen dimensions)	low< 87 high> 87

The 2013 ELN recommendations[2] for patients with CML in CP, AP and BP are illustrated in Table 4 and 5.

Table 4. 2013 ELN recommendations for patients with CML in CP for first, second and next treatment lines

First line
-Imatinib or Nilotinib or Dasatinib -HLA typing for patient and siblings in case of unfavorable prognosis at diagnosis (high score, major cytogenetic abnormalities in Philadelphia positive cells)
Second line and in case of first line TKI intolerance
-every other TKI approved in first line (Imatinib, Nilotinib or Dasatinib)
Second line in case of warning zone or failure to Imatinib in first line
-Dasatinib or Nilotinib or Bosutinib or Ponatinib - HLA typing for patient and siblings
Second line in case of case of warning zone or failure to Nilotinib in first line
-Dasatinib or Bosutinib or Ponatinib - HLA typing for patient and siblings; in case of no HLA matching sibling, allogeneic transplant from unrelated HLA matching donor should be considered
Second line in case of case of warning zone or failure to Dasatinib in first line
-Nilotinib or Bosutinib or Ponatinib - HLA typing for patient and siblings; in case of no HLA matching sibling, allogeneic transplant from unrelated HLA matching donor should be considered
Third line in case of warning zone or failure or/and intolerance to second generation TKI
-any unused TKI ; allogeneic transplant of all eligible patients
Any treatment line in case of T315I mutation
-Ponatinib - HLA typing for patient and siblings; in case of no HLA matching sibling, allogeneic transplant from unrelated HLA matching donor should be considered

Table 5. 2013 ELN treatment recommendations for patients with CML in AP and BP

TKI naïve of AP and BP cases at diagnosis	-Imatinib 400 mg bid or -Dasatinib 70 mg bid or -Dasatinib 140 mg od -search of related or unrelated HLA matched donator and then -allogeneic transplant for all BP patients at diagnosis and AP patients without optimal response to TKI -chemotherapy should be used for disease control before allogeneic transplant
TKI treated CML cases who progress from CP to advance phase (AP and BP)	any unused TKI before progression (Ponatinib in cases of T315I mutation) and then allogeneic transplant for all eligible patients - chemotherapy should be used for disease control before allogeneic transplant

For assessing hematological, cytogenetic and molecular responses during TKI treatment, the following definitions are used (Table 6).

Table 6. Definitions of TKI treatment response in CML

Type of response	**Definition**
Complete hematological response (CHR)	Normal blood counts and differential
Minor cytogenetic response (mCyR)	35-90% Philadelphia chromosome positive metaphases
Partial cytogenetic response (PCyR)	1-34% Philadelphia chromosome positive metaphases
complete cytogenetic response (CCyR)	0% Philadelphia chromosome positive metaphases
Major molecular response (MMR or MR^3)	Reduction ≥ 3-log a *BCR-ABL1* mRNA or *BCR-BL1* ≤ 0.1%
4 molecular response ($MR^{4.0}$)	*BCR-BL1* ≤0.01% or undetectable *BCR-ABL1* IS with number of *ABL1*control- gene copies ≥10.000 or ≤ 32.000
5 molecular response ($MR^{5.0}$)	*BCR-BL1*≤0.001% orundetectable*BCR-ABL1* IS with number of *ABL1*control- gene copies ≥100.000
Completemolecular response (CMR)	negative result assessed by qRT- PCR

mRNA = messenger RNA; PCR = polymerase chain reaction; For all types of cytogenetic responses at least 20 metaphases should be analyzed (Radich JP. *Blood*.2009;114:3376-81). For assessing cytogenetic and molecular responses during TKI treatment,the 2013 ELN recommendations[2] are used (Table 7).

Table7. 2013 ELN recommendations for cytogenetic and molecular responses

At diagnosis	-conventional cytogenetic exam using banding technique for assessment of bone marrow metaphases -peripheral blood FISH exam in case of Philadelphia chromosome negativity by conventional exam and in case of suspicion of variant and cryptic translocations -qualitative and quantitative RT-PCR for type and level of *BCR-ABL1*transcript (p210)
During TKI treatment	- quantitative RT-PCR for *BCR-ABL1* (p210) transcript level detection according to international scale every 3 months until MMR (*BCR-ABL1≤0,1%* or MR[3]) and then every 3-6 months and/or -conventional cytogenetic exam using banding technique for assessing at least 20 bone marrow metaphases at 3, 6 and 12 months until CCyR and then every 12 months; after CCyR, RT-PCR exam could be the only technique used for disease monitoring
Warring zone	-more frequent cytogenetic and molecular monitoring - cytogenetic monitoring in case of myelodysplasia or cytogenetic abnormalities in Ph negative cells with chromosome 7 anomalies
Progression	-qRT-PCR exam -mutational analysis - conventional cytogenetic exam - peripheral blood and/ or bone marrow immunophenotyping exam in BP

According to the 2013 ELN recommendations for CML[2], some therapeutic targets were established for assessing individual response to TKI treatment (Table 8 and 9).

Table 8. 2013 ELN recommendations for assessing response to first line of TKI

	Optimal response	**Warning zone**	**Failure**
At diagnosis	not applicable	-high risk or -major cytogenetic abnormalities in Ph positive cells	not applicable
At 3 months	-*BCR-ABL1* ≤ 10 % and/or -Ph+ ≤ 35%	-*BCR-ABL1* ≥ 10 % and/or -Ph+ = 36-95%	-nocomplete hematologic response and/or -Ph+ > 95 %
At 6 months	-*BCR-ABL1* ≤1% and/or -Ph += 0 %	-*BCR-ABL1*> 1-10 % and/or -Ph += 1-35 %	-*BCR-ABL1*> 10% and/or -Ph + > 35 %
At 12 months	-*BCR-ABL1* ≤ 0,1%	-*BCR-ABL1*> 0,1- 1 %	-*BCR-ABL1*> 1 % and/or -Ph+ >0 %
After 12 months and at any time	-*BCR-ABL1* ≤ 0,1%	-cytogenetic abnormalities in Ph negative cells (monosomy 7 or 7q deletion)	-loss of CHR -loss of CCyR -loss of MMR -mutations - cytogenetic abnormalities in Ph positive cells

Table 9. 2013 ELN recommendations for assessing response to second line TKI in case of Imatinib failure

	Optimal response	**Warning zone**	**Failure**
At diagnosis	Not applicable	-without or loss of CHR during Imatinib or -without cytogenetic response at first line of TKI or -high risk	not applicable
At 3 months	-*BCR-ABL1* ≤10 % and/or -Ph+ < 65%	-*BCR-ABL1*> 10 % and/or -Ph+ = 65- 95%	-no CHR or -Ph+ >95 % or -new mutations
At 6 months	-*BCR-ABL1* ≤10% and/or -Ph + < 35 %	-Ph + = 35- 65 %	-*BCR-ABL1* > 10% and/or -Ph + > 65 % and/or -new mutations
At 12 months	-*BCR-ABL1* ≤1% and/or -Ph + =0 %	-*BCR-ABL1*> 1- 10 % and/or -Ph += 1- 35 %	-*BCR-ABL1*> 10% and/or -Ph + > 35 % and/or -new mutations
After 12 months and at any time	-*BCR-ABL1* ≤0,1%	- cytogenetic abnormalities in Ph negative cells (monosomy 7 or 7q deletion) or -*BCR-ABL1*> 0,1%	-loss of CHR or -loss of PCyR and CCyR or -loss of MMR -new mutations - cytogenetic abnormalities in Ph positive cells

In rare cases, primary and secondary resistance to Imatinib are the identified mechanisms which explain failure and loss of initial response to Imatinib. Themechanisms involved in Imatinib resistance are resumed in Table10.

Table10. Mechanisms involved in Imatinib resistance

BCR-ABL1 independent mechanisms	*BCR-ABL1* dependent mechanisms
Patient related -reduced compliance to treatment	*BCR-ABL1*supraexpression -mutations in ABL kinase domain
Pharmacological -reduced intestinal absorption -drug interactions -bound to plasmatic proteins	
Leukemic cells - leukemic cell heterogeneity -low levels of transports (hOCT1) -high levels of exporters (ABCB1, ABCG2)	
Dormant stem cells	
Clone evolution	
SRC supraexpression	

In 45-50% cases, the detection of a mutation could explain the resistance to treatment. The most frequent mutations are resumed in Table 11.

Table11. Frequent identified mutations

Mutation	Resistance mechanism to treatment
T315I F359V F317L	direct implication of Imatinib biding
Q252H/R G250E E255K M244V Y253F/H L387M	prevents modification of P loop conformation
H396R	prevents modification of activation loop conformation
E355G V379I M351T	prevents modification of C-terminal end conformation

For our department practice, the ELN recommendations for mutation analysis[2] are synthesized in Table 12.

Table12. ELN recommendations for mutation analysis

At diagnosis:
Only in AP and/or BP patients
During first line Imatinib treatment:
In patients in warning zone and/or failure
In cases with increasing *BCR-ABL* transcript level which is followed by MMR loss
During second line treatment with Dasatinib or Nilotinib:
In cases of failure

In our department, TKI treatment switch according to mutation is resumed in Table 13.

Table 13. Treatment options according to identified mutation

T315I
Allogeneic transplant or inclusion in clinical trial
V299L, T315A si F317L/V/I/C
Nilotinib
Y253H, E255K/V si F359V/C/I
Dasatinib
Any other mutation
Imatinib in higher dose or Dasatinib or Nilotinib

At the moment, allogeneic transplant represents the only cure for CML[6]. With long-term optimal TKI treatment results and high morbidity and mortality rates of allogeneic transplant, the latest procedure is recommended as second or third line treatment option for selected patients. According to ELN recommendations, allogeneic transplant in CML is indicated for: all BP patients and some AP patients at diagnosis, in warning zone or failure after first TKI line, patients in warning zone or failure after second generation TKI and patients with T315I mutation. Before first and second generation TKI availability, European Group for Blood and Marrow Transplantation (EBMT) has established a CML prognostic score for evaluation of patients with allogeneic transplant indication according to 5 variables which is still accurate in TKI era. According to EBMT score, patients with CML were classified as low if score = 0-2, intermediary if score = 3-4 and high if score = 5. In TKI era, the accuracy of this score was confirmed by 5 year survival of 70% in low score category and of 40% in high score category.

Table 14. EBMT score for CML

Factor	Score
CML phase	
CP	0
AP	1
BP	2
Age	
< 20 years	0
20- 40 years	1
>40 years	2
Donor type	
related	0
unrelated	1
Time from diagnosis until transplant	
< 12 months	0
>12 months	1
Donor-host sex compatibility	
woman-man	1
man- man	0
woman-woman	0
man-woman	0

The use of TKI after allogeneic transplant is still controversial and should be considered after special assessing of individual cases or in case of relapse.

Conclusions:

In our department, first TKI line is decided according to patient`s co-morbidities, phase and risk scores at diagnosis. The second TKI line is decided according to patient`s co-morbidities, CML phase at treatment failure, presence or absence of additional cytogenetic abnormalities, presence or absence of mutation status, primary or secondary treatment resistance and HLA matched donor availability. Allogeneic transplant in CML is indicated for all BP patients and some AP patients at diagnosis, in warning zone or failure after first TKI line, patients in warning zone or failure after second generation TKI and patients with T315I mutation. Imatinib is recommended as first line to all patients with significant morbidities, classified as low and intermediary risk, in different doses according to phase at diagnosis, no major route additional cytogenetic abnormalities and no atypical BCR-ABL1 transcripts. Nilotinib and Dasatinib are recommended as first line to patients without significant morbidities (see indications and contraindications), with different doses according to phase at progression, classified as intermediary and high risk and in search of HLA matched donor availability.

For CP patients with CML who for any reasons fail Imatinib in first line, HLA matched donor availability is searched, Nilotinib and Dasatinib are recommended in second line according to CML phase at treatment failure, presence or absence of additional cytogenetic abnormalities, presence or absence of mutation and primary or secondary treatment resistance. For CP patients with CML who for any reasons fail Nilotinib in first line, HLA matched donor availability is searched, Dasatinib is recommended in second line according to CML phase at treatment failure, presence or absence of additional cytogenetic abnormalities, presence or absence of mutation and primary or secondary treatment resistance. For CP patients with CML who for any reasons fail Dasatinib in first line, HLA matched donor availability is searched, Nilotinib is recommended in second line according to CML phase at treatment failure, presence or absence of additional cytogenetic abnormalities, presence/or absence of mutation and primary or secondary treatment resistance. In our department, Bosutinib and Ponatinib are not available for use in second or third or as bridge to allogeneic transplant except clinical trials. For CP patients with CML who for any reasons fail first two TKI lines, HLA matched donor availability is searched, allogeneic transplant is discussed and inclusion in clinical trials with new TKI is recommended as bridge to transplant.

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Intramural hematoma and small intestinal occlusion in a patient with severe Haemophilia A- case report: Prophylaxis - cost / benefit?

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Abstract

***Background**: Haemophilia A is a rare bleeding disorder (~1:5000-10.000 males), most often inherited (X-linked recessive disease), characterized by factor VIII deficiency. The optimal management of the haemophilia patients is complex and extremely costly, requiring the use of replacement therapy with coagulation factor concentrates during the entire life span as well as the treatment of chronic and acute complications.*

Although haemophilia A affects only a small portion of the population, the costs of care are disproportionately high and more often the resources are limited. There are few comparative studies regarding the economic benefits of prophylactic vs on demand therapy, but the clinical and social advantages of the first are well known.

***Case presentation**: We report the case of a 37 years old patient diagnosed with severe haemophilia A, chronic debilitating arthropathy following repetitive episodes of joint bleeding, poor quality of life and no work opportunities. He is receiving on demand therapy with factor VIII concentrates.*

During a period of 1 year the patient was hospitalized for almost 180 days (in our Haematology Department and the local hospital). The total cost were extremely high. Only in our hospital he received almost 362.000 units of factor VIII. We do not have the exact amount of units used at the local hospital, but we can estimate a total of 140.000 (an average of 2000U/day x 70 days). So we have a total of ~500.000 units in 1 year. When referring to haemophilia, the health authorities take into account strictly the cost of the antihaemophilic factor. But, there are always additional costs related to: hospitalization days, surgery procedures, lab tests, imaging tests (ultrasounds, CT scans), medical supplies and concomitant medication. And they never calculate the social costs and the impact on quality of life.

Many of the patients' complications could have been prevented by using a prophylactic regimen with its clinical and economic benefits.

***Key words**: severe haemophilia A, on demand therapy, prophylactic therapy, intramural hematoma*

Case report

We present the case of a 37 years old patient, diagnosed with severe haemophilia A (Factor VIII level <1%) from birth, chronic hepatitis B and C infection (secondary to plasma derived products received at younger age), repetitive hemarthrosis with progressive deterioration in joint function (elbows, ankles, knees) and walking impairment due to constant pain, muscle atrophy and stiffness. He is under episodic therapy (on demand) with Factor VIII concentrates, either from the local hospital or from our Comprehensive Haemophilia **Care Centre**.

He is unemployed and receives financial support from the state. His quality of life and social integration are poor and the burden on the health system is high.

In Jan 2010, after frequent episodes of intestinal bleeding, especially after non-steroidal anti-inflammatory drugs (taken for the chronic joint pain), he was diagnosed with Crohn disease and was put on treatment with Salazopyrin, with temporary improvement.

Due to the lack of peripheral venous access, we had to implant a port-a-cath device in order for the patient and nurses to be able to administer the treatment in case of a bleeding event.

In 2015 the patient suffered repeatedly from

intestinal bleedings caused by the intake of pain medication (NSAIDs) for chronic hemarthrosis. He needed blood transfusions in several cases, iron supplements and a large amount of Factor VIII concentrates.

On 31 Jul 2015 he was involved in a small car accident caused by a sudden forced brake and an abdominal trauma due to the tightened seat belt (worn across the low abdomen, rather than in a normal fashion). In hours he begun to have local pain and swelling. As soon as he got home he self-administered a large amount of Factor VIII (3000 U) and the next day he went to the local hospital.

On 04 Aug he was referred to our Haemophilia Centre for further treatment, with severe anaemia (Hb 6.7 g/dl), APTT 75.2 sec, Fact VIII level 1%, no inhibitors and low iron levels. The CT-scan (Fig.1) showed a large intra-abdominal hematoma (12.9/5.5 cm) just behind the anterior abdominal wall, with mass effect upon the intestinal tract and the bladder; no active bleeding was detected.

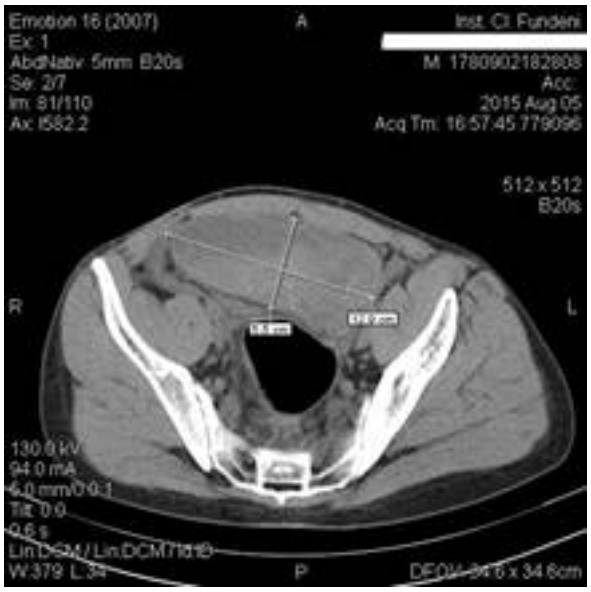

Fig.1 (large intra-abdominal hematoma)

He received blood transfusions, IV iron and Factor VIII 3000 U bid then 1000U/8h, with clinical and biological improvement. The patient was released from hospital after 5 days, with home replacement therapy.

On 04 Sep he returned to our hospital with fever, low abdominal pain and swelling, pallor, sweats. A new CT scan (Fig.2) revealed recent hematomas into the anterior abdominal wall (5.4/4.2cm and 1.4/1.2cm) and a slight decrease of the previous hematoma (now measuring 10.5/5.5cm). He remained hospitalized for 19 days, receiving broad spectrum antibiotics, Factor VIII 2000U bid. The recovery was slow and the patient went home.

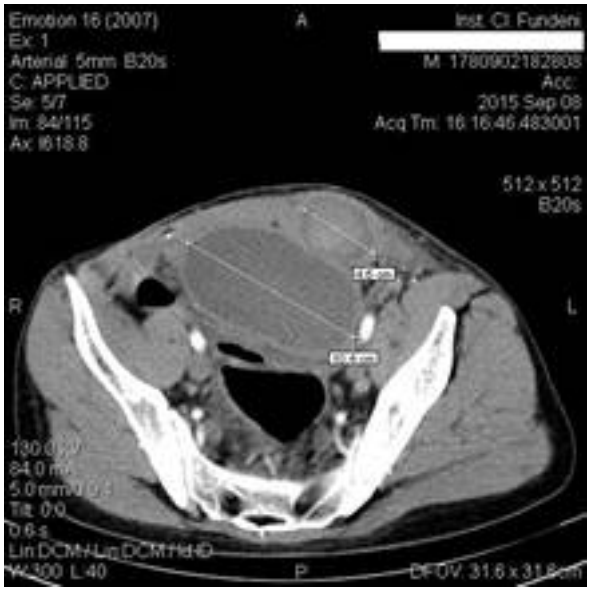

Fig.2 (recent hematoma into the abdominal wall)

After just two weeks, on 13 Oct, he was hospitalized at the local medical centre for generalized abdominal pain, lack of appetite and weakness. The clinical status did not show any improvement after 4 days and the patient begun to develop signs of bowel obstruction with absence of faeces and flatulence during the previous 24 hours, so he was sent to our clinic for further investigations and therapy. The CT scan revealed a recent hematoma (5.3/4.1cm) located in the left iliac fossa, between the intestinal loops witch appeared slightly distended; one nodular image into the enteral lumen (blood clot?) (Fig.3 and 4).

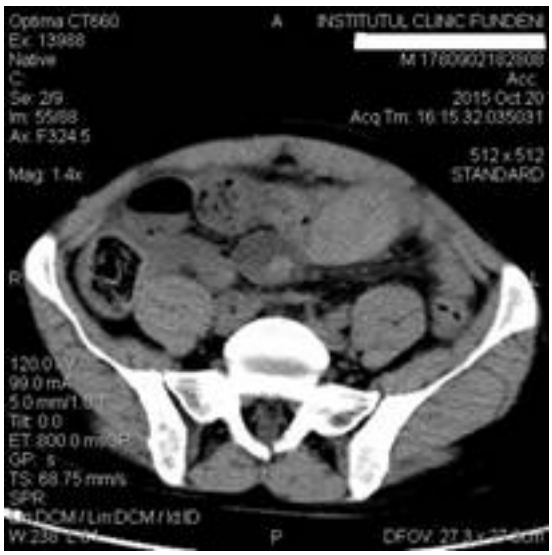

Fig.3 (Native)

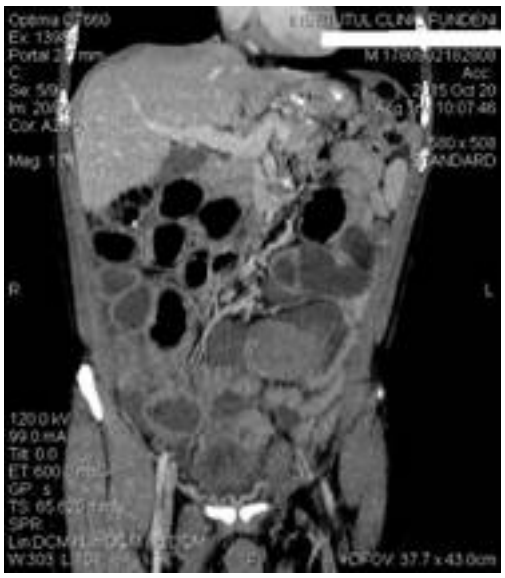

Fig.4 (Coronal view)

He was repeatedly examined by surgeons who recommended a conservatory approach due to the patient's relatively good condition (no vomiting or pain, without signs of peritoneal irritation) and the risks associated with an open surgery. A naso-gastric tube was inserted and the patient received factor concentrates (2000U bid), IV fluids and food supplements. The patient's condition did not improve. Repeated abdominal X-scans (Fig. 5 and 6) and CT scans (Fig.7) showed constant progressive distention of the small bowel and jejunal hydro-aero levels.

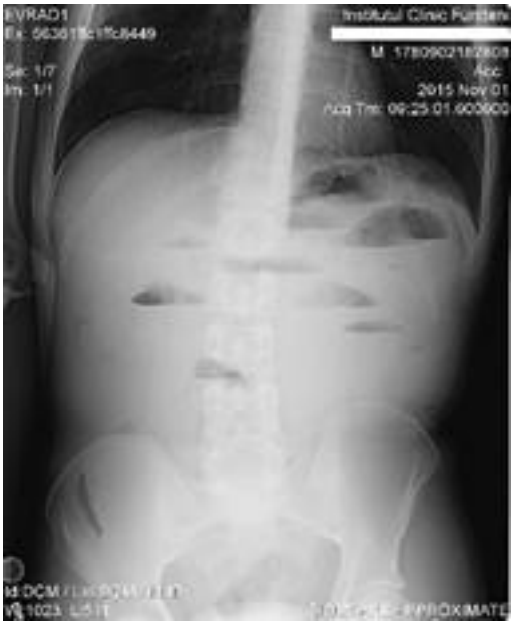

Fig.5 (01 Nov)

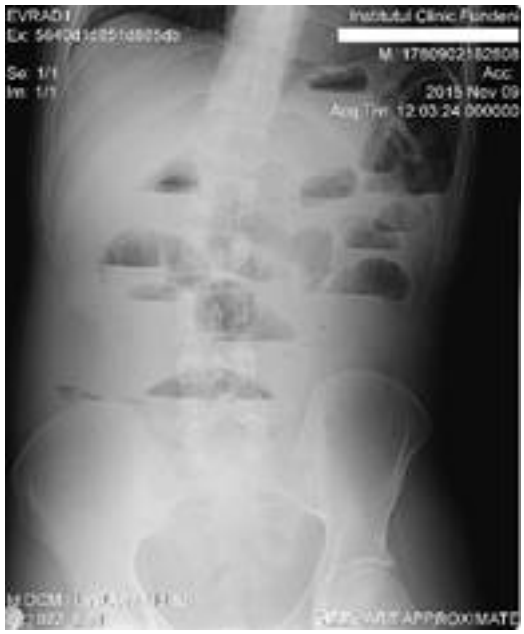

Fig.6 (09 Nov)

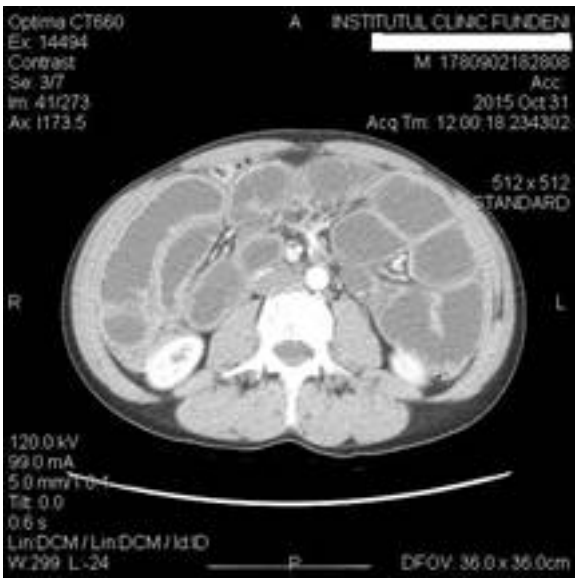

Fig.7 (bowel distention and food stasis)

On 10 Nov his general status begun to worsen, he started vomiting (at 1-2 hours after fluid/food intake) and to experience abdominal distension and pain. He was re-examined by the surgeons and he was transferred to the Surgery Department later that day.

On 11 Nov he entered surgery with a Factor VIII level of ~100%. Intra-operatory, the cause of the bowel obstruction was discovered: an intramural hematoma. A segmented enterectomy was performed. After the surgery the patient received 2000 U/8h of Factor VIII concentrates 7 days, broad spectrum antibiotics, IV fluids. Although his Fact VIII levels were kept high (>60%), he presented several blood losses through the drain tubes, requiring factor supplementation and transfusions. The lab tests showed an increased INR (2.8) and decreased prothrombin activity (28%), low albumin level (1.6 mg/dl), low cholesterol (25 mg/dl), a sign of hepatic function impairment. He received fresh frozen plasma (4U/day) with slowly improvement of the coagulation. His bowel function was restored 5 days after surgery. The patient was transferred back to our Haematology Department on 19 Nov and continued the treatment with Factor VIII 2000 U bid (with dose supplementation at every sign of bleeding).

The patient's recovery was slow, with repetitive haemorrhage from the incision, fever and anaemia. He was later discharged on 17 Dec, after 59 days of hospitalization. He continued home treatment with Factor VIII concentrates.

Discussions:

People with severe Haemophilia A suffer from recurrent and spontaneous bleeding episodes (joint and muscle, central nervous system, digestive tract) with acute and chronic complications: progressive arthropathy and joint damage, anaemia, neurologic deficiency, compartment syndrome, pseudotumors and even death. The therapy consists of intravenous administration of Factor VIII concentrates.

There are 5 types of treatment [2]:

1. Episodic (on demand) - given at the time of bleeding
2. Primary prophylaxis – regular continuous treatment started before the second large joint bleed and before the age of 3 years
3. Secondary prophylaxis – regular continuous treatment started after 2 or more large joint bleeds but before the onset of joint disease
4. Tertiary prophylaxis - regular continuous treatment started after the onset of joint disease to prevent further damage
5. Intermittent (periodic) prophylaxis – given to prevent bleeding for short periods of time (during and after surgery)

Today, the prophylactic therapy is gaining terrain (especially in children), with well-known clinical benefits. There are several trials that already demonstrate the effectiveness of prophylactic therapy in childhood, in preserving the joint function. Its use in adult patients is limited by the cost of treatment and the frequency and inconvenience of administration (more often in patients with poor venous access). Two trials in adult patients with severe Haemophilia A (SPINART and POTTER) are ongoing in USA and Italy.

The benefits of prophylaxis [1] [4]:

1. Fewer joint bleeds – 3 x times less likely to have a joint bleed
2. Less arthropathy and reduced disability
3. Fewer muscle bleeds
4. Reduced risk of severe bleeds (cerebral)
5. Fewer hospital admissions and less frequent monitoring
6. Less time off work/school – it reduces the productivity loss
7. Less joint surgery
8. Fewer bleeds/year
9. Decreased severity of bleeding episode, with reduced amount of factor used/bleed
10. Enables patients to enjoy an active life, including the possibility of physical activities, regular school attendance, social, and work opportunities
11. Better quality of life

The current situation in Romania is the following: the children under 18 years with severe haemophilia (Factor VIII levels <1%) are receiving prophylactic treatment with recombinant factor concentrates (25 U/Kg, 3 days/week, without the possibility of dose individualisation according to the patient's pharmacokinetic parameters and bleeding phenotype). Adult patients are on episodic therapy (on demand), whenever a bleeding episode occurs. Most of them already have chronic arthropathy and advanced disabilities, chronic hepatitis B or C (from plasma derived transfusions), frequent haematomas or digestive tract haemorrhages. For patients with inhibitors the situation is quite severe: the possibility of treatment is scarce and only available for a bleeding episode. ITI or prophylaxis are not possible due to high costs.

The best care for these patients is provided in Haemophilia Treatment Centres or Haemophilia Comprehensive Care Centres (like the one in our hospital), where a multidisciplinary team is always available for the right treatment of the haemophiliacs, 24 hours a day, 7 days a week.

Intramural haematoma of the intestine is a very rare complication of this disease [5]. According to the literature, there are 33 described cases of haemophilia patients with intramural hematoma of the gastrointestinal tract from 1964 to this day. Some of the main causes are trauma, intestinal pathological conditions and the widespread use of nonsteroidal anti-inflammatory drugs (NSAIDs) in the treatment of chronic arthropathy. The most likely event is the rupture of a terminal artery as it enter the muscle layer of the intestinal wall. The haemorrhage dissects between the muscle layers and the muscularis mucosae. The best diagnostic tool is the computer tomography due to its high sensitivity.

The initial approach should be conservative, non-surgical [6]. Surgery is reserved for cases in which a complication appears (perforation, ischemia, intra-abdominal bleeding and untreatable obstruction).

There are many diseases characterised by a deficiency of a certain protein (thalassaemia, diabetes, Addison, hypothyroidism) and whose treatment consists in regular replacement therapy rather than "on demand". Some rare disorders like paroxysmal nocturnal haemoglobinuria, Fabry, Gaucher benefit from prophylaxis – although more expensive than in haemophilia – so why in haemophilia things should be any different? [1]

In adults it is reasonable to continue the prophylaxis when started in childhood, in order to maintain healthy joints and good quality of life [3]. Patients who already have advanced arthropathy also may benefit from tertiary prophylactic regimen, with fewer joint bleeds and less disability, better social life, improved general condition, less need of antalgic therapy (especially NSAIDs).

Conclusions:

Our case certainly demonstrates the chronic and acute complications of an insufficient therapy in haemophilia and the high costs associated with every bleeding episodes left undertreated. Every intestinal bleed was triggered by the use of NSAIDs taken for the frequent (almost continuous) joint pain; secondary hepatitis B and C made his liver more prone to function deficiency in special cases (general anaesthesia, post-operative status, chronic medication); his poor quality of life is determined by his chronic and disabilitating arthropathy, walking impairment and frequent intestinal

bleeding with secondary anaemia.

If the patient would have been on prophylactic therapy (25 U/Kg x 3/week), the amount of Factor concentrate used in this year (~500.000 units) would be sufficient for ~111 weeks (~2 years). The social and clinical benefits would clearly overpass the cost barrier.

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Acute Basophilic Leukemia with AML1-ETO and IgG lambda monoclonal gammopathy - CASE REPORT

and review of the data presented in the literature

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Abstract

Acute basophilic leukemia is a type of acute myeloid leukemiain which differentiation is to basophils. It is a very rare disease, representing less than 1% of the cases of AML. We present herein the case of a 61-year-old man referred to our Center in July 2013 for asthenia, fatigue, tegument pallorerythematous edematous pruritic plaques on the limbs shin.. The diagnosis of acute basophilic leukemia was set because of cytomorphological, immunohistochemistry, immunophenotyping characteristicsof myeloid blast cells, bone marrow tryptase positivity. Molecular biology showed the presence of AML1-ETO in the absence of c-kit mutation and Philadelphia chromosome. The patient received initial treatment with hydroxycarbamide, then chemotherapy with doxorubicin and citarabine, type "3 + 7" cycle , to which we added H1, H2 receptor antagonists with good response. The bone marrow aspirate performed in 14-day counted 6-7% blasts, followed by a similar reinduction and 3 consolidation cycles with age-adjusteddose cytarabine. The patient is in complete remission and currently ETO AML-1-negative.

Key-words*: acute basophilic leukemia, AML1-ETO*

Introduction

Acute basophilic leukemia is a type of acute myeloid leukemiain which differentiation is to basophils. It is a very rare disease, representing less than 1% of the cases of AML. Although it was first described more than 75 years ago, there are no well-known / consistent diagnosis criteria. This type of leukemia was first described in 1906 by Jaochim in two patients with basophilia and clinical features of AML. In 1982 it was described as a type of acute basophilic leukemia by Wick and hiscolleagues, after which similar cases were reported by others.

Optical microscopy describes circulating blast cells as medium-sized, with a large round nucleus, bilobed, incised, with dispersed chromatin and 1-3 nucleoli, a reduced quantity of cytoplasm, which is basophilic and contains numerous granules that are positive in metachromatic stains, mature basophils being rare.

Electron microscopy shows the presence of structures that are characteristic to basophils precursors at the level of the granules. The most specific cytochemical feature is a colouring of the granules with toluidine blue. Blasts are PAS positive and can be negative for MPO and SBB.

Cutaneous biopsy (for cases showing cutaneous manifestations) shows a diffuse arrangement of younger elements.

On Immunophenotyping both basophils and mast cells show a myeloid phenotype (CD 33, CD13) that is not specific. The presence of CD9, CD25 is characteristic of basophilic differentiation. The detection of abnormal mast cells expressing CD117, CD25 and mast cell tryptase differentiate acute basophilic leukemia from acute mast cell leukemia.

Most cases of acute basophilic leukemia evolved from chronic phase myeloid leukemia, but there were cases described without Philadelphia chromosome. Because CML involves Philadelphia chromosome (t(9;22)(q34;q11), with the same breakpoint –q(34), on chromosome 9, as AML associated with translocation t(6;9), and both types are associated with bone marrow basophilia, it is possible that a gene at the breakpoint on chromosome 9 is involved in basophilopoiesis.

This type of leukemia is not described as a distinct

type of leukemia in the FAB classification due to its rarity. In the recent WHO classification of myeloid malignancies, acute basophilic leukemia was integrated distinctly and defined as an acute myeloid leukemia in which primary differentiation is to basophils.

AML1-ETO, t(8;21)(q22;q22) is present in 8% AML patients under the age of 50 and 3% AML patients over 50 years of age . It is associated with loss of Y chromosome in men and X chromosome in women in more than half of the patients.

Acute myeloid leukemia with t(8;21)(q22;q22) and inv16(p13.1;q22)/t(16;16)(p13.1;q22) are categorized as core binding factor (CBF) – AML due to the implication of subunit alpha and beta genes in chromosomal translocations. CBF – AML represent approximatively 15% of AML cases. C-KIT mutations are detected in CBF – AML with a frequency that varies between 12-48%. Although CBF leukemias are considered to have a favourable prognosis, there are subsets of patients that present an early relapse and a reserved prognosis. NCCN guides defined acute leukemias that associate t(8;21)/inv(16) to c-KIT mutation as having intermediate risk; however ELN guides make no recommendations to those who present c-KIT association. Adults with t(8;21) and inv(16) present mutations mainly at exon 17 and 8 respectively. A large number of leukocytes at diagnosis and higher level of AML-ETO transcripts in adult patients with t(8;21) and c-KIT mutation have been associated with a high cumulated risk of relapse after 2 years, and leukemia-free survival reduced to 2 years and reduced overall survival probabilities. CALGB study and a german multi-case study indicated that a reduced number of platelets at diagnosis was also associated with reducedoverall survival and a short period without relapse. Unlike adult patients, paediatric patients associating such mutations did not present such an influence.

Clinical exam: Patients diagnosed with acute basophilic leukemia may present symptoms caused by histaminemia: urticarial skin lesions, peptic ulcer, organomegaly, lytic lesions, anaphylaxis.

The prognosis of acute basophilic leukemia is generally reserved, the disease being resistant to treatment, but there are cases cited in the literature that associated AML1-ETO translocation with a favourable prognosis.

Treatment is similar to other types of AML, and the prognosis is reserved, associated with chemoresistance. Treatment should include the addition of H1 and H2 receptor antagonists because of the risk of anaphylaxis, gastric acid hypersecretion, peptic ulceration and gastrointestinal bleeding caused by the degranulation of the basophilic cells.

Case report:

We present herein the case of a 61-year-old man referred to our Center in July 2013 for asthenia, fatigue, tegument pallor.

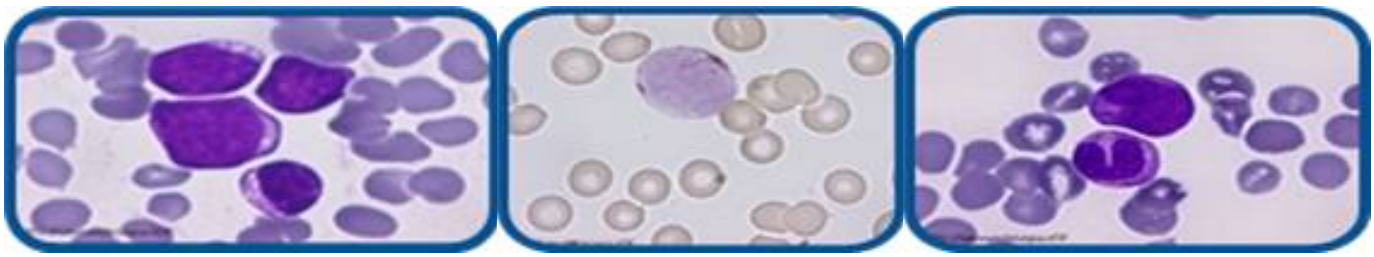
Clinical exam: good general condition, without fever, with tegument pallor, erythematous edematous pruritic plaques on the shins, without organomegaly, cardiorespiratory function stable.

Laboratory tests: Hb= 10.6g, Ht 31.2%, L= 66240/mmc T= 9000/mmc, MCV= 96.6fL (Blast cells= 42, Pro=1, Mi=4, Mt=2, N=1, S=5, B=40, L=5) . Cytochemical tests: POX positive 40% in peripheral blood, Auer-rod positive.

Hepatic, renal tests and coagulation tests were in normal ranges,AgHBs – negative, Ac antiHCV – negative,HIV – negative; HTLV – negative.

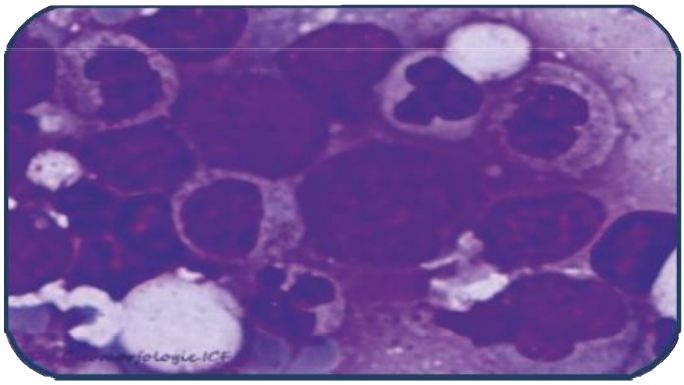
Bone marrow aspiration could not be performed because of the bone marrow infiltration; therefore a core biopsy and phenotyping of peripheral blood were performed.

Peripheral blood smear: Polymorphous blast cells, sparse, with round nuclei and Auer rods, some of which with incised, lobular nuclei. Basophils of all stages, including basophiloblasts.



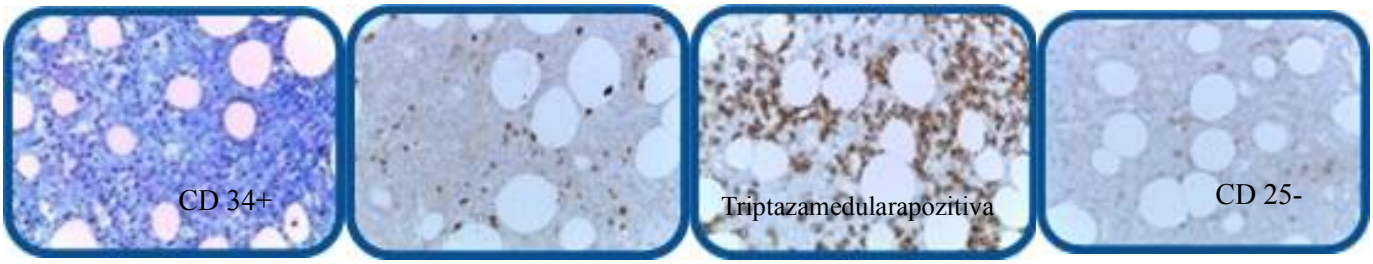
Bone marrow smear

Blast cells of medium/large size with a round nucleus, fine chromatin, some of them with nucleoli and a small quantity of basophilic cytoplasm. Sparse



Bone marrow biopsy

IHC bone marrow tests: CD25 (lymphocyte marker activated and mast cell tumor) positive in very sparse small lymphocytes(3%), CD34 positive (40%) and CD68 positive in histiocytesbut also in frequent mononucleated groups (mast cells?). Tryptase-positive bone marrow.

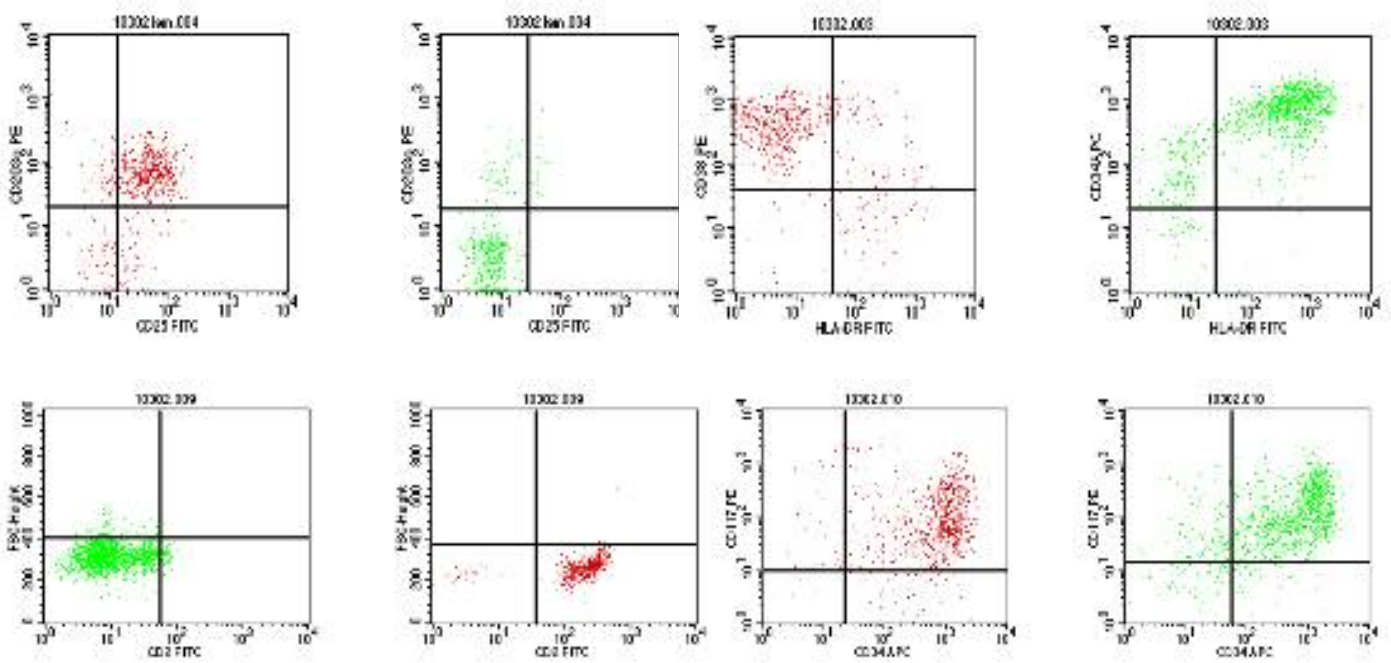


Imunophenotyping:

Immunophenotypic analysis detected: 70% of peripheral blood cells with small-average internal complexity fully expressing CD117, CD34, cyMPO, CD33, CD38, CD13 weak, CD123, CD71weak, CD16 +/- and which is divided into two subpopulations: a subpopulation (50%) which co-expressed HLA-DR, CD19, and a subpopulation (25-30%) with small SSC and CD45 more positive - that co-expressed CD203c, CD22, CD2, CD25 (marker for mast cell disease). All blast cells are CD117+.

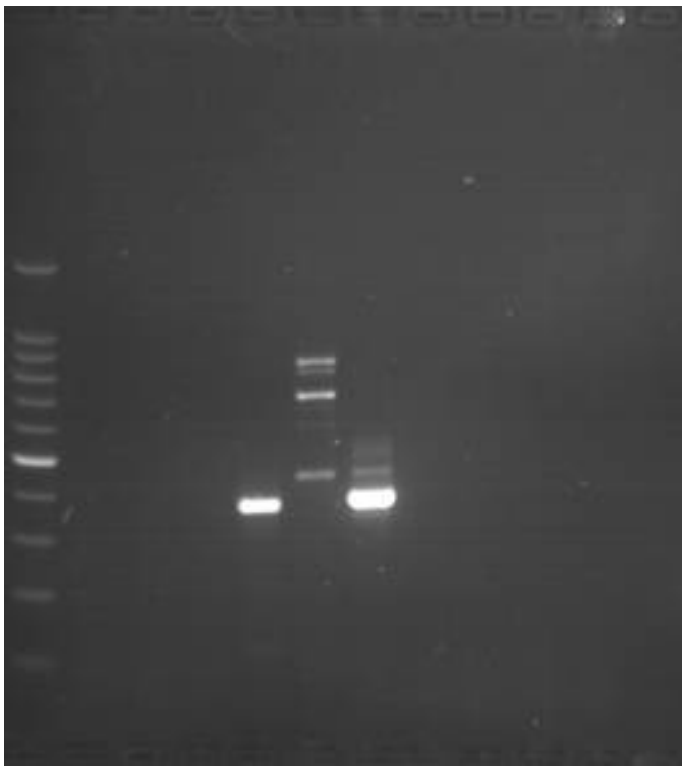
Green: basophilic blasts: HLA DR+, CD203c-, CD19+, CD2-, CD25-, CD15+

Red: pathological mast cells: DR-, CD203c+, CD2+, CD25+, CD15- (markers for neoplastic cell).



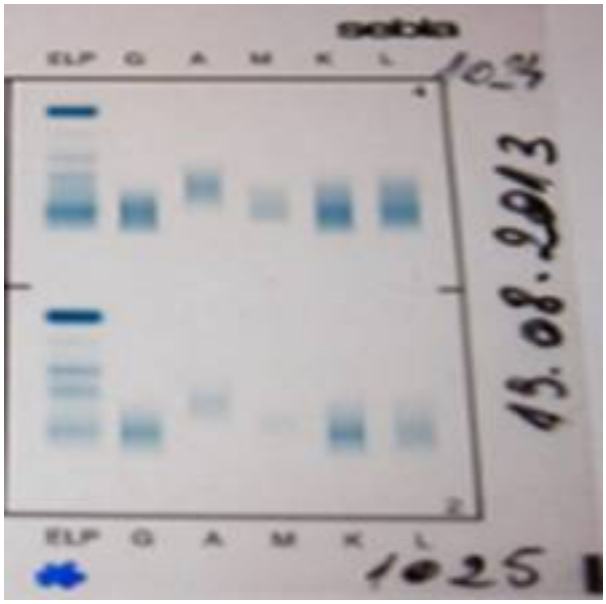
Molecular biology tests evidenced AML1-ETO presence. **Does not associate C-KIT or BCR-ABL**.

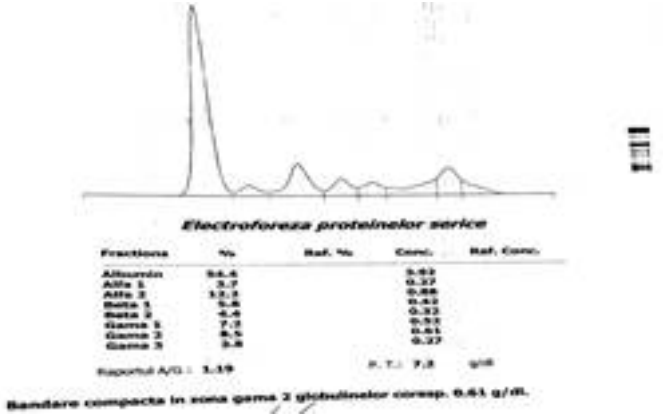
Multiplex PCR Method.
Weight marker every 100 pb.
Ampliconul AML1-ETO is at 395pb.
Tube 1-PrimersforAML1-ETO



Serum electrophoresis with immunofixation

Serum protein electrophoresis shows in the compact peek in beta2 globulin area. Serum immunoglobulin dosage (including IgE) were normal, except for IgG level, which was high. Serum immunofixation revealed compact peek in the range 2 globulin identified with antiserum anti heavy gamma chain and antiserum anti kappa light chain (fig .)





The patient received cytoreductive therapy with Hydroxycarbamide to reduce the risk of tumor lysissyndrome, tandaftertgattype "3 + 7" treatment cycle.

Because in acute basophilic leukemia serious complications are associated with heavy histamine release from the basophils undergoing degranulation, the treatment associated antagonists of H1 and H2 receptors and Dexamethasone.

Day 7 medular aspirate showed reduced cellularity and the one performed on day 14, 6-7% blasts (myeloblasts), basophils <1%.

The patient subsequently received another "3 + 7" cycle–that resulted in complete remission, followed by 3 age-adjusted dose cytarabine consolidations and managed to maintain complete remission until now. Molecular biologyperiodically performed revealed disappearance AML-ETO transcript. Currently 3 years have passed after diagnosis and the patient is still in complete remission, AML-ETO negative.

Serum protein electrophoresis reveals no changes in monoclonal gammopathy, whose significance has not yet been clarified.

DISCUSSION:

Patient diagnosed with acute leukemia with double population, of both basophils and mast cells, diagnosis that is associated with poor prognosis in literature, which expresses the mutation AML-ETO (+) associated with favorable prognosis in the absence of c-Kit mutation. Pacient associated IgG monoclonal gammopathy, whose prognostic value is unknown at this time.

Differential Diagnosis:

A differential diagnosis with chronic myeloproliferative disorders must be made, acute mast cells leukemia, systemic mastocytosis, myelomastocytic leukemia or systemic mastocytosis with acute myeloid leukemia.

In this case, the cytomorphological characteristics, the myeloid phenotype of the blasts cells, with positive CD34, cyMPO, HLA-DR (+), CD203c (-), CD19 (+), CD2 (-), CD25 (-), CD15 (+), the absence of Philadelphia chromosome, the presence of AML1-ETO translocation, the positive medullar tryptase, excluded blast crisis of chronic myeloid leukemia, acute lymphoblastic leukemia, acute myeloid leukemia, acute mast cells leukemia.

Until now, there are just a few cases of acute leukemia with basophils described in the literature.

Ritu Gupta, Paresh Jainand Mona Anand (Rotary Cancer Hospital Institute, All India Institute of Medical Sciences New Delhi, India) presented in 2004 in the American Journal of Hematology a case of a 6-year old patient with acute leukemia with basophils with t(8; 21), following AML-M2 with basophilia. No mention was made with respect to c-KIT mutation. The patient went into complete remission after first treatment with cytarabine and daunorubicin, after which he received a high-dose cytarabine consolidation, maintaining complete remission at 4 months after chemotherapy.

Peterson et al (Department of Laboratory Medicine and Pathology, Hennepin County Medical Center, University of Minnesota) published in August 1991 an article presenting 8 cases of acute leukemia with basophils selected from 455 cases of acute leukemia examined under electron microscopy.

Cases that presented ultrastructural evidence of blast differentiation to basophils were considered basophilic acute leukemia cases. In 6 of these cases the diagnosis could not be made after an optical microscopy exam, therefore an ultrastructural analysis had to be performed by electron microscopy. For all 8 cases the ultrastructural analysis revealed immature basophilic granules, which showcases the differentiation to basophils of myeloid blasts. Theta granules were evidenced in 4 patients, which is characteristic to early differentiation. In 3 of the 8 cases electron microscopy revealed proof of mast cell differentiation. Cytogenetic testing of these cases revealed association with Philadelphia chromosome in 3 patients, but none of the 3 patients has had a history of chronic myeloid leukemia; none of the other cases presented chromosomal abnormalities known to be associated with acute myeloid leukemia such as t(6;9) or abnormality at the level of 12p, 2 of the patients had normal karyotypes. None of the patients presented clinical signs caused by the degranulation of basophils and excess histamine, which was evidenced by Wick et al in 3 out of the 4 cases of acute leukemia with differentiation to basophils. The cases reported by Wick et al were diagnosed based on optical microscopy exam. Over 10% of the blasts were granular, MPO negative, but metachromatic with toluidine blue. The ultrastructural analysis revealed differentiation to basophils in one of the patients.

So Youn Shin et al presented the case of a 72-year old patient with acute leukemia with basophils and 7 monosomy in all analyzed metaphases, c-KIT negative, Philadelphia (-). At diagnosis the patient had patches and petechiae, erythematous skin, dyspnea and an altered state. He received treatment with cytarabine and idarubicin with partial response.

XIAO-HUA LUO (Chongquing Medical University, China) et al described the case of a 65-year old patient, who presented fatigue, vertigo, melaena lasting for 2 weeks and cutaneous rash on hands and legs. Blasts were MPO and PAS positive and negative for alpha-naphthyl-acetate esterase. Bone marrow smear presented atypical immature basophils and mature basophils. Blast cells were positive with toluidine blue stain. Cytogenetic testing revealed abnormal karyotype. C-KIT D816V and chromosome Philadelphia were negative. Phenotyping revealed the presence ofCD11b, CD13, CD22, CD25, CD33, CD123. The patient was treated with "3+7" chemotherapy treatment with partial response after which the rash reoccurred. Therefore, the patient was treated afterwards with doses ofidarubicin and cytarabine, with partial response. Three weeks after hospital release, the patient succumbed following infectious complications.

Most reported cases of acute basophilic leukemia seemed at first accelerated phase chronic myeloid leukemia, Philadelphia chromosome positive, with a high number of mature and immaturebasophils. Moreover, cases of myeloproliferative diseases in which differentiation was made to basophils have also been described.

Most cases reported in the literature do not provide details on treatment response and followup or data on survival. However, our patient had an excellent response to chemotherapy and represents a case of long-term survival. This is probably due to t(8; 21)

association, and the absence of c-KIT mutation or Philadelphia chromosome.

The case described above (i.e. acute leukemia with basophils AML1-ETO positive), showcases the importance of immunohistochemistry and molecular biology, but also immunophenotyping, in establishing diagnostic certainty. These tests have allowed matching thetreatment with the risk group and the association of complementary measures so as to avoid complications. AML 1 - ETO also indicates a favourable prognosis in this rare type of acute leukemia.

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Secondary thrombotic thrombocytopenic purpura or Atypical onset of Systemic Lupus Erythematosus – challenge of diagnosis: case presentation.

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Abstract

Primary or secondary thrombotic thrombocytopenic purpura (TTP) is a severe disorder and without treatment it is fatal.

We present the case of a 37 years old female, with no prior medical history, presented in our hematology ward with fever, fatigue and nausea. The clinical exam revealed pallor and multiple bruises. On the preliminary laboratory tests we discovered severe anemia and thrombocytopenia, moderate schistocytosis on the peripheral blood smear, elevated lactate dehydrogenase (LDH) and a high level of fibrin degradation products. The preliminary diagnosis for this patient was thrombotic thrombocytopenic purpura. Treatment was started immediately with corticosteroids and plasma exchange (PEX). Due to the lack of response and the declining neurological state of the patient, the question was if there is an underlying cause for the TTP. After taking the immunology panel of tests and seeing the positive antinuclear antibodies (ANA) and anti-Smith antibody, the final diagnosis was systemic lupus erythematosus with hematologic, neurologic and immunologic manifestations.

Keywords: *thrombotic thrombocytopenic purpura (TTP), systemic lupus erythematosus (SLE), plasma exchange (PEX), corticosteroids.*

Introduction

Thrombotic thrombocytopenic purpura (TTP) is a syndrome caracterised by mostly fatal outcome without rapid diagnosis and treatment. TTP is a distinct entity of the thrombotic microangiopathic disorders. The syndrome is characterized by thrombocytopenia, hemolytic anemia with microangiopathic mechanism, multiple thrombosis in microvascularization of different organs. The etiology is complex and it includes infections, autoimmune disorders such as SLE, solid and hematological malignancies and very rare, congenital conditions. The clinical panel depends on the etiology. The positive diagnosis for TTP is made on five criteria: fever, thrombocytopenia, microangiopathic hemolytic anemia, neurological manifestations and renal involvement (not mandatory). The paraclinical features are represented by anemia with reticulocytosis, schistocytes on the peripheral blood smear (due to the section of the erythrocytes as they are passing through the thrombi in the vascular system), severe thrombocytopenia, elevated lactate dehydrogenase (LDH), varying degrees of acute kidney injury. In medical practice, there are incomplete forms, but for the diagnosis are mandatory: fever, neurologic disorders and hemolytic anemia with schistocytes, thrombocytopenia and low level of ADAMTS13 <10%. (1, 2,3,4,5)

The discovery of ADAMTS13 enzyme was an important step in discerning the pathogenesis of TTP. ADAMTS13 is a zinc–protease that cleaves the von Willebrand factor, blocking the activation, adhesion and aggregation of the thrombocytes to the endothelium. In TTP the activity of ADAMTS13 is below 10%. The low activity is either due to the insufficient production by the responsible gene (congenital TTP) or by low function because of autoantibodies (6).

TTP is classified as primary TTP and secondary

TTP. Secondary TTP appears associated with many conditions such as autoimmune diseases, drugs, pregnancy, malignancy, malignant hypertension or in stem cell transplant recipients. Treating the underlying cause is the main condition, but there are cases in which plasma exchange is effective. The association of SLE with TTP is rare (0, 5% of secondary TTP) (1) thus the incidence is unknown due to lack of studies (2,3,4,5). The treatment consists in plasma exchange, corticosteroids and immunosuppressive drugs. The overall response to PEX in secondary TTP may vary (50-70%)(3,4), meanwhile in primary TTP it exceeds 80% responses.(3,4)

Case presentation

A 37 years old Caucasian female was admitted in our clinic in December 2014 with a 2 weeks history of fever, nausea and vomiting. The pacient had no history of drug abuse, toxic exposure.

The physical examination revealed a febrile (38.6ºC), confused patient with skin pallor and generalized bruises. No other abnormalities were found in the physical examination. No signs of pregnancy were revealed by gynecological examination.

The blood count showed: Hb=7 g/dL (normal range 12-16g/dl); WBC=10820/mmc (normal range 4000-8000/mmc), Plateletes =6000/mmc (normal range 150.000-450.000/mmc); Ret=14% (normal range 0.5-2.5%). The peripheral blood smear showed frequent microcytes and moderate schistocytosis (Fig. 1)

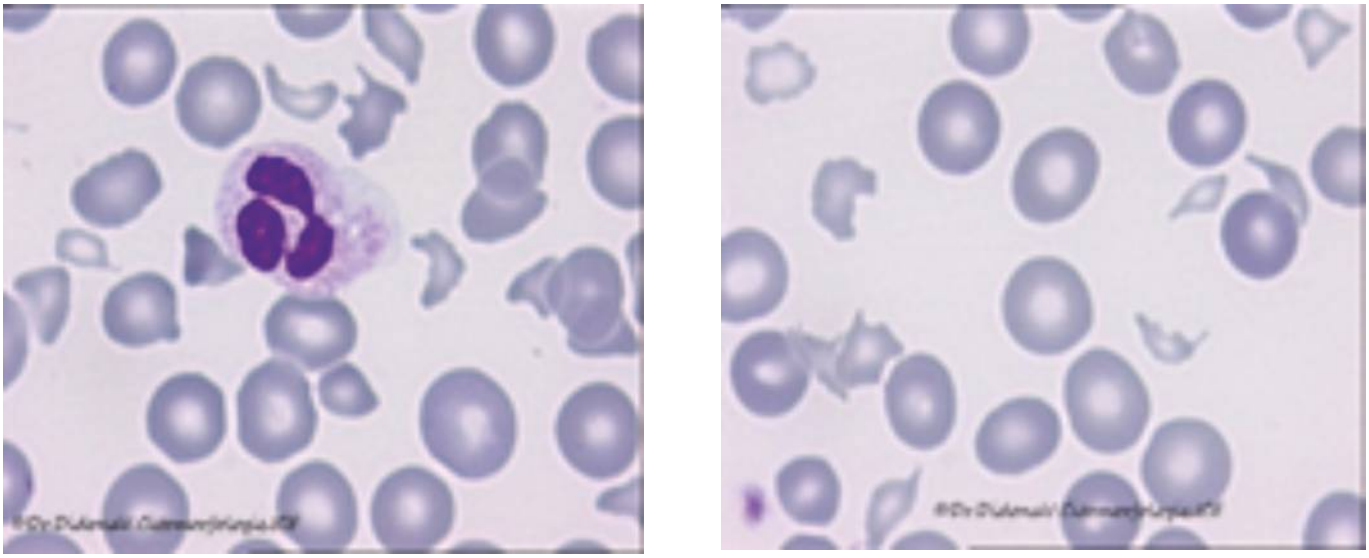


Fig 1: Peripheral blood smear, MG Giemsa 100x – Schistocytosis (courtesy of D. Vasilache)

Blood clotting tests showed normal levels of fibrinogen (263 mg/dl), with increased levels of fibrin degradation products: D-dimers >20000 µg/L (normal range <500µg/L), intense positive fibrin degradation products (FDPs). Blood workup also revealed total bilirubin of 5.3 mg/dl (normal range 0.3 – 1.9 mg/dl) with indirect bilirubin of 3.9 mg/dl (normal range 0,2-0.7 mg/dl), LDH=1984 IU/L (normal range 100-200 IU/L) and a positive Coombs test for complement component 3 (C3). No biological signs for renal failure were observed. No arguments for solid malignancy were found. The bone marrow aspiration and bone marrow biopsy reveraled hypercellularity (80/20) with erythroid hyperplasia with moderate macrocytic appearance and about 4-5% interstitial, reactive lymphoid infiltrates with B cells CD20 positive.

The preliminary diagnosis for this patient is thrombotic thrombocytopenic purpura based on clinical criteria (fever, overall clinical worsening state, confusion) and paraclinical criteria (thrombocytopenia, microangiopathic hemolytic anemia).

The differential diagnosis is to be made with immune thrombocytopenia , disseminated intravascular coagulation (DIC) of other causes(sepsis), hemolytic –uremic syndrome, Evans syndrome.

Treatment with dexamethasone and plasma exchange daily was started with an initial good response. The clinical state of the pacient improved, but no other improvement was seen regarding thrombocytopenia, anemia or LDH.

On december 25th, the patient developed seizures followed by coma in 24 hours for which she needed to be transfered in an Intensive Care Unit (ICU). The neurological examination and imagistic tests (cerebral CT scan) revealed no neurological condition.

Due to the worsening state of the pacient and the lack of response to PEX and corticosteroids, an underling cause of TTP was suspected.

The paraclinical examination was completed with an extensive autoimmune antibody screening (table 1).

29th of December 2014	Results	Normal range
ANA detect	8.8	0-1.1 U/mL
Anti ds-ADN screen	17.8	0-25 U/mL
Anti SS-A (Ro)	>200	0-25 U/mL
Anti SS-B (La)	13.7	0-25 U/mL
Anti beta2-Glycoprotein screen	4.6	0-10 U/mL
Anti- Cardiolipin screen	7.9	0-10 U/mL
Anti- IF	1.5	<6 U/mL
Anti - LKM1	2.6	0-10 U/mL
Anti Phospholipid screen	3.6	0-10 U/mL
Anti SLA	4.2	0-10 U/mL
ENA – screen	7.6	0-1 U/mL
Parietal	51.7	<10 U/mL

Table 1 – Autoimmune screening results. The results were positive for ANA detect, Anti SS-A, Parietal and ENA screen.

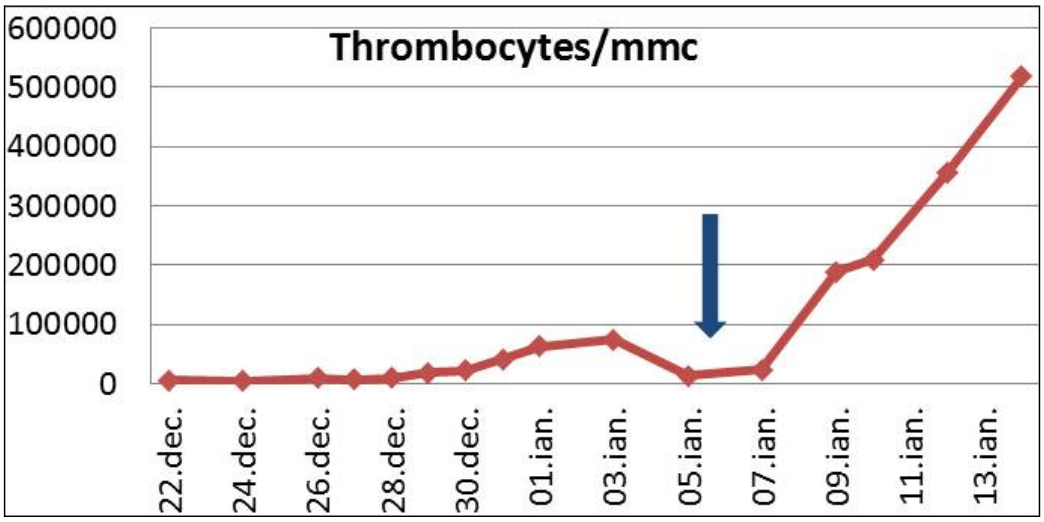
According to the American College of Rheumatology Classification Criteria for systemic lupus erythematosus (SLE) (…) a positive diagnosis was made based on the following four criteria: neurological disorders (seizures, coma); hemolytic anemia, thrombocytopenia and positive immunology for Anti SS-A and ANA.

After consulting with a fellow rheumatologist, a 5 days course of immunosuppression with methyl-prednisolone 500 mg/ day was started and the plasma exchange procedure was stopped because of inefficiency. The complete diagnosis was SLE with severe manifestation, probably due to endothelial damage of microvascularization. The outcome was rapidly favorable.

After 5 days of steroids, the thrombocyte count was normal (>150000/mmc) and the overall clinical state of the patient greatly improved. The patient was discharged one week later with the following parameters:

- Hb=9,3 g/dL; WBC=8850/mmc; Plt=518000/mmc;
- TB=0,5 mg/dL; LDH=216 mmol/L

In the figure below is shown the dynamic of platelets count from admission to discharge ; we observe a improvement of platelets count after initiating specific treatment for the underlying cause of TTP.



The arrow shows the moment before and after treatment with methylprednisolone.

Discussions

The association between TTP and SLE is very rare (?01% of all cases)(…). TTP and SLE can be difficult to diagnose because of the overlapping features.

The red cell fragmentation syndrome can appear in SLE secondary to the endothelial lesions, due to the microthrombosis from the antiphospholipidic syndrome. Anemia can have also an autoimmune component which presents with leukoerithroblastic picture in the peripheral blood smear, elevated LDH, but schistocytes are not a frequent finding(<20%). Thrombocytopenia can also have an autoimmune mechanism. In the case we described, the hemolysis and the microangiopathy coexisted (positive Coombs test and fragmented red cells). The neurologic disorders can appear in LES as a manifestation of the vasculitis, but can also be a TTP manifestation.

The differential diagnosis can be made to other microangiopathies like Hemolytic – Uremic Syndrome (SHU) ; the pacient had no abdominal pain, bloody diarrhea, manifestations of the hemorrhagic enterocolitis due to Escherichia coli O157:H7. Evans syndrome alone or as epiphenomena of SLE could be another variant for the patient but the presence of fragmented erythrocytes makes less probable this diagnosis . (4, 5, 8)

For objective reasons, we weren't able to determine ADAMTS13 activity. This is the main strong parameter for the differential diagnosis of thrombotic microangiopathy versus immune disorders secondary to SLE.

The first sign that there was an underlying cause for TTP was the lack of response to PEX. It is known that PEX is not very efficient in SLE with TTP associated. The measurement of ADAMTS13 activity could have established the final diagnosis but the procedure is very elaborate and it is not recommended to wait for the results , because of the patient's clinical state .

According to the American College of Rheumatology (ACR) Classification Criteria four of the following criteria are needed to have a positive diagnosis of SLE: skin involvement (malar rash, discoid rash, oral ulcers), photosensitivity, non-erosive arthritis, serositis, renal abnormalities including proteinuria, neurologic disorders, positive immunological markers (anti-ds DNA or anti-Smith antibody and high Antinuclear antibody (ANA)) and hematological abnormalities. The hematological abnormalities include hemolytic anemia, lymphopenia and thrombocytopenia. (7)

We faced an unusual case of secondary TTP due to SLE in a young female patient, with no prior medical history and with a poor response to plasma exchange therapy. After initiating high immunosuppressive treatment, rapid clinical and paraclinical improvement were seen.Even in the absence of explicit criteria of diagnosis of TTP and low level of ADAMTS13 activity, the decision for beginning PEX therapy is crucial. It is recommended that in the case of TTP with severe neurological abnormalities, the PEX and immunosuppression need to be started simultaneously as soon as it is possible. Many patients who matched the diagnostic criteria for TTP do not have severe ADAMTS13 deficiency. Therefore, in daily practice the therapeutical attitude is based on the gravity of the clinical state of the patient (4).

The positive outcome for this case was made possible by a multidisciplinary collaboration between neurology, rheumatology, anesthesiology and hematology specialists.

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Parvovirus B19 infection and hemophagocytic syndrome in a patient with renal transplant

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Abstract

Parvovirus B19 infection may have life-threatening complications in immunosuppressed patients.

This is a case of a 49 year old patient who had a kidney transplant and immunosuppressive therapy afterwards, that presented to our Clinic for anemia and thrombocytopenia associated to encephalitic neurologic symptoms and fever. The bone marrow biopsy raised the suspicion of Parvovirus B19 infection, and PCR-AND Parvovirus of 186.000.000 copies/ml confirmed the diagnosis. The association with hypofibrinogenemia, hypertrigliceridemia, hiperferritinemia, medullar hemophagocytosis, bicytopenia (anemia and thrombocytopenia), especially in the context of Parvovirus B19 infection in an immunossupressed patient rises the question of an associated hemophagocytic syndrome. The encephalitic symptoms and the IRM images of nonspecific ventriculomegaly (although described in other cases of Parvovirus B19 infection) may be related to the infection, in the absence of PCR testing of the cerebrospinal fluid.

Symptoms improved and viremia reduced after lowering immunossupression and IVIG treatment, but neurologic symptoms persisted (seizures). Kidney function deteriorated and she restarted hemodialysis.

Keywords: *Parvovirus B19, hemophagocytic syndrome, immunosuppression, renal transplant*

Introduction:

Parvovirus B19 infection can be trivial, self-limiting, in the immunocompetent patient, but, in certain cases it can have life-threatening complications: transient aplastic crisis in patients with hereditary hemolytic anemia (sudden drop in hemoglobin).

In immunossupressed, the virus may persist in the blood flow and can give chronic pure erythroid aplasia. Lowering the immunossupression in the kidney transplant patient, allows the immune system to respond against the virus.

Platelets are not directly affected by Parvovirus B19, but it can still give sight thrombocytopenia.

A rare complication of Parvovirus infection is hemophagocytic syndrome that is associated to potentially life-threatening cytopenias.

Case presentation: A 48 years caucasian female patient was admitted in May 2015 to a local infectious disease ward with flapping tremor, memory loss and intermittent dysarthria. She also had vesperal fever, chills and low visual acuity. The patient was known to have familial juvenile hyperuricaemic nephropathy for which she received a renal transplant in april 2012. She was on immunosuppressive treatment with prednisone, mycophenolate mofetil and tacrolimus and antihypertensive treatment with Beta2-blockers and Calcium channel blockers.

On physical examination she was confused, had a tremor and hemiballistic movements. Moderate pallor, peripheral edema, a heart rate of 120bpm and a blood tension of 170/90 mmHg were noted.

The general laboratory tests showed moderate normochromic normocytic anemia (hemoglobin levels of 8 g/dl) with no other changes on the complete blood count and elevated VSH 54mm/h (normal range <20 mm/h) and C reactive protein 46,9ng/ml (normal range 0-5mg/l). Normal levels of procalcitonin and a slight elevation of 446mg/dl in the fibrinogen levels (normal range 200-400mg/dl). She also had a creatinine value of 1.9mg/dl (normal range 0.6 to 1.1 mg/dl), urea value of

85.2mg/dl (normal range< 43mg/dl) and a low creatinine clearance value of 30ml/min (normal range 88-128 mL/min for healthy women).

During her stay in the infectious disease ward she further developed vertigo, confusion and visual hallucinations.

A lumbar tap was performed which showed 20 mononuclear cells per microliter (normal range 0-5cells/microliter) with normal glucose and protein levels in the spinal fluid.

She underwent a cerebral MRI scan that showed ventriculomegaly, microangiopathic subcortical ischemia and demyelination in the frontal, parietal and posterior cortices.

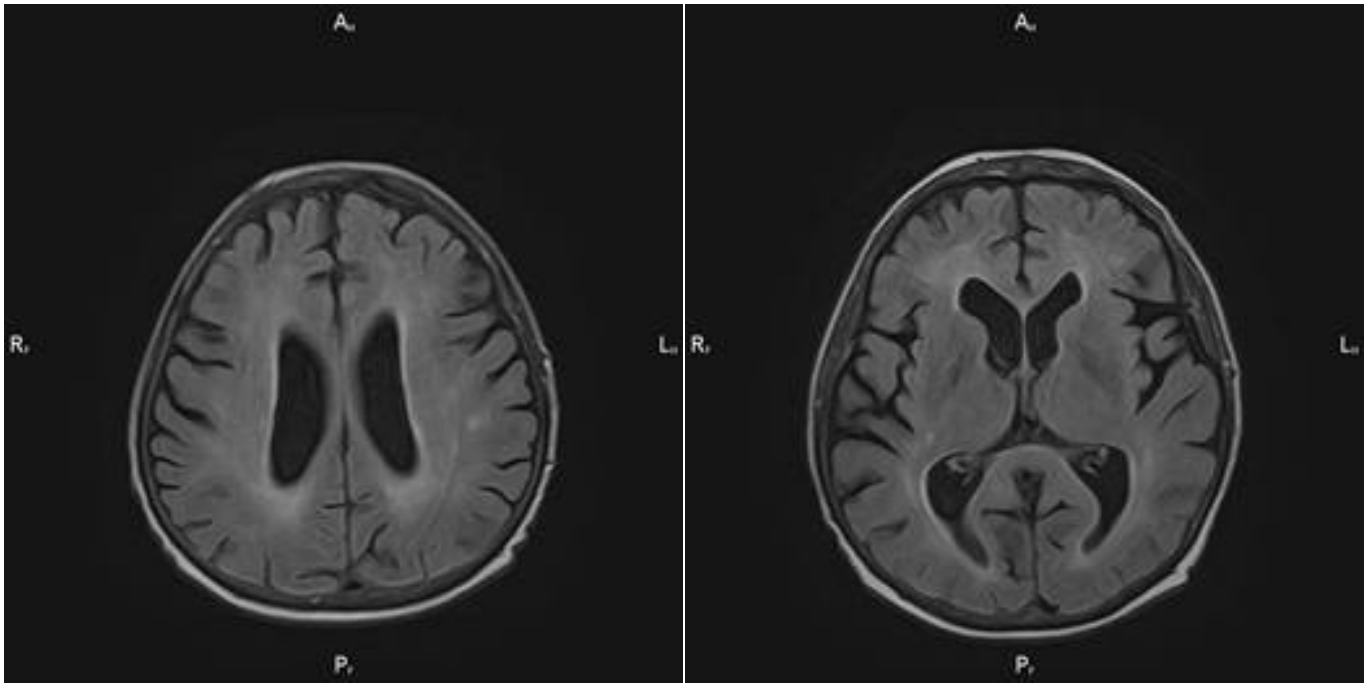

Figure 1 Cerebral MRI showing ventriculomegaly.

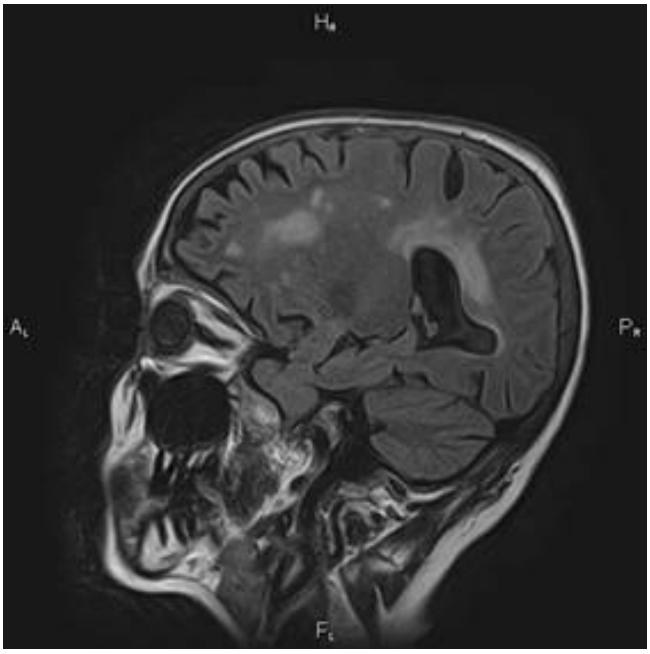

Figure 2. Cerebral MRI showing demyelination in the frontal, parietal and posterior cortices.

The serological testing for cytomegalovirus, HIV, Toxoplasma gondii, Cryptococcus neoformans, rubella, syphilis and Coxiella burnetii was negative.

Nonetheless the patient received antiviral treatment with acyclovir and ganciclovir, antibiotherapy with ampicillin and moxifloxacin , antituberculosis treatment and dexamethasone.

Soon after the patient showed a degradation of the neurological status developing seizures. She was admitted in the intensive care unit for treatment and monitoring. In the ICU the immunosuppressive treatment was stopped for eleven days.

The lab tests showed thrombocytopenia 70000/microliter (normal range 150000-45000/mmc), hypofibrinogenemia 128mg/dl, elevated D-dimer value 1591 ug/l (normal range <500ug/l), elevated C reactive protein 51.8ng/ml and procalcitonin 3.48ng/ml (normal range< 0.5ng/ml) and a rise in the creatinine and urea levels (Cr. 3mg/dl, Urea 257mg/dl)

At this point the treatment consisted of moxifloxacin, meropenem, teicoplanin, doxycycline and fluconazole.

Immunosuppression was restarted with methyl-prednisolone, mycophenolate mofetil and tacrolimus. Under immunosuppressive treatment the reactive C protein and procalcitonin levels normalized but a worsening of hypofibrinogemia was seen (Fibrinogen

51mg/dl). No renal improvement was noted with the reintroduction of the immunosuppressive drugs.

In June 2016 the patient underwent dialysis in a nephrology ward were beside low platelets and fibrinogen levels high levels of ferritin and triglycerides were noted.

She was admitted in our Hematology ward in June 2015 under the diagnosis of hemophagocytic syndrome.

Normochromic normocytic anemia (Hb. 7.7g/dl) with low reticulocyte count 0.0029 cells/mmc (normal range 30000-120000/mmc) and thrombocytopenia (Plt.33000/mmc) was seen on the complete blood count.

Laboratory test showed an elevated creatinine and urea levels (Creatinine 1.96mg/dl, Urea 163mg/dl) and a high triglycerides 350mg/dl (normal range150 to 199 mg/dl) and ferritin > 1650ng/ml (normal range13-150ng/ml) levels.

A bone marrow aspiration and biopsy was performed.

The bone marrow tap described a hypocellular marrow, with severe dyserythropoiesis, polymorphic megakaryocytes with low platelets production, frequent active macrophages containing ferric deposits and cellular debris.

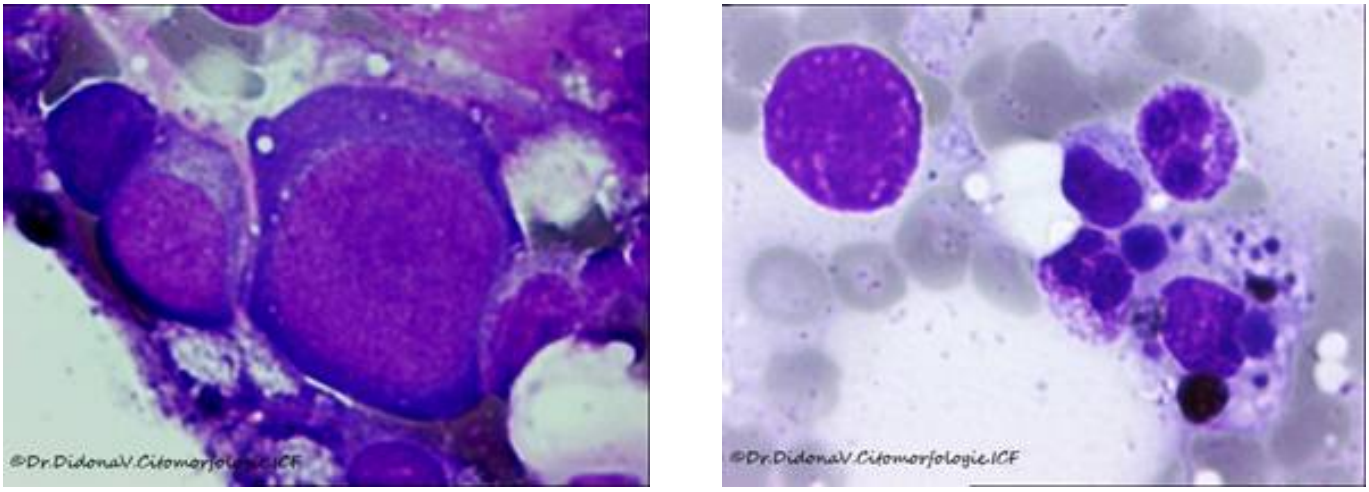

Figure 3. Bone marrow aspiration-gigantic erythroblast and multinucleated erythroblasts. (Courtesy of doctor D. Vasilache)

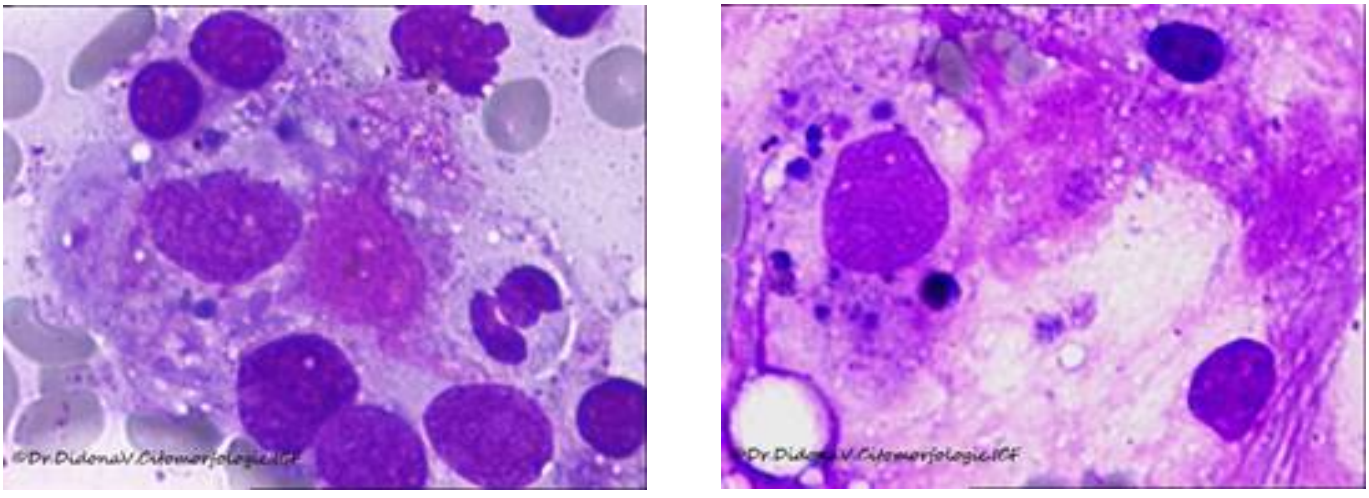

Figure 4. Bone marrow aspiration- active macrophages with embedded erythroblast, neutrophils ferric granules. (Courtesy of doctor D. Vasilache)

Fig.2. Bone marrow aspiration

The bone marrow biopsy showed hypocellularity with severe erythroblastopenia, megaloblastoid cells with nuclear inclusions, scarce megakaryocytes and relatively frequent siderophages. A suspicion of parvovirus B19 was made on the bone marrow biopsy.

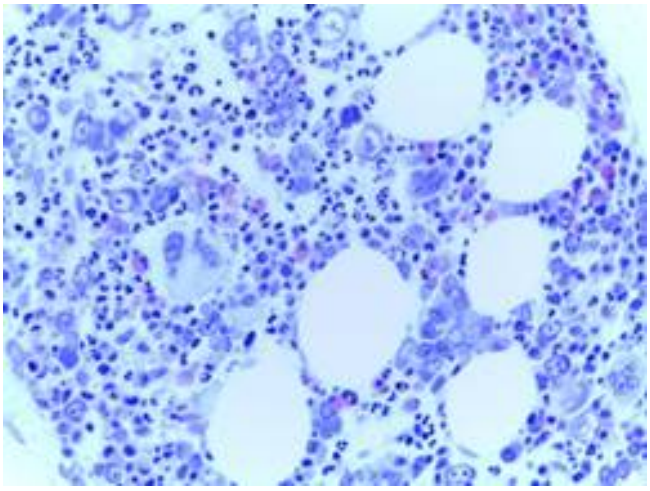

Figure 5. Bone marrow-Giemsa 40X Hypocellularity. Megaloblastoid cells with nuclear inclusions (Courtesy of doctor C. Dobrea)

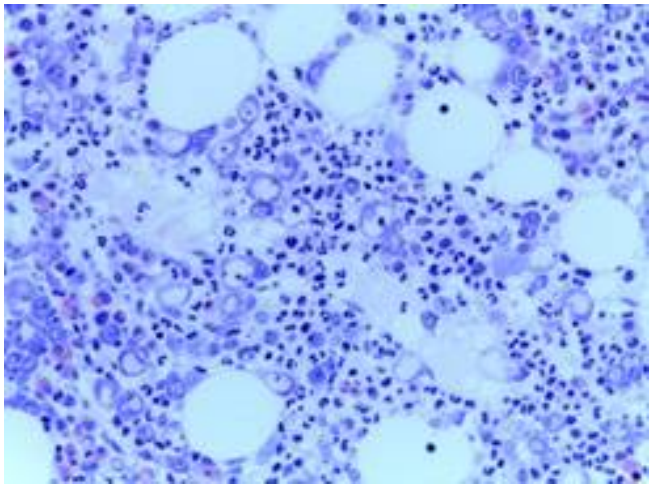

Figure 6. Bone marrow-Giemsa 40X Erythroblastopenia and megaloblastoid cells with nuclear inclusions. (Courtesy of doctor C. Dobrea)

Parvovirus B19 viral load of 186.000.000 copies/ml was detected through PCR.

The patient was diagnosed with aplastic crisis due to parvovirus B19 infection and possibly an associated hemophagocytic syndrome (hypofibrinogenemia, hypertriglyceridemia, hyperferritinemia, medullary hemophagocytosis, anemia and thrombocytopenia on CBS in the context of a viral infection in a immuno-compromised patient)

Treatment with dexamethasone and cyclosporine was started with a favorable response. An increase in the hemoglobin 10,7g/dl and platelets levels 118000/mmc (starting hemoglobin 7.7g/dl and platelets 33000/mmc) and reticulocyte count was seen after 10 days of treatment. Later treatment with intravenous immunoglobulins was started and the patient made a good recovery and was discharged.

After 3 months of the incurring infection the patient was admitted in the renal transplant ward of our hospital with confusional state and epileptic grand mal crisis.

A cerebral MRI scan was performed that showed no new lesions in the brain.

The blood count showed normochromic normocytic anemia with hemoglobin 9.6g/dl and normal platelets 217000/mmc. The lab test showed elevated creatinine 2.15mg/dl levels and normal fibrinogen and triglycerides. Parvovirus B19 viral load count of 367000 copies/ml and a BK viral load of 104 copies/ml. No active JCV or CMV infections were detected on PCR testing. The patient was started on chronic dialysis and anticonvulsant treatment. She was later discharge in good condition.

Discussion:

Parvovirus B19 infection:

Parvovirus B19 is the only human pathogen from the Parvoviridae family, and it has high affinity for the bone marrow. The viral replication takes place only in the human erythroid progenitor because they express the B19 cellular receptor (globoside = P antigen).[1]

In immunocompetent patients, B19 causes erythema infectiosum ("fifth disease" = slapped cheek rash). It is a childhood exanthema; in adults it can be associated with acute symmetric polyarthropaty. In hemolytic anemia patients it can be the cause of transient aplastic crisis.[1]

In immunossupressed, persistent parvovirus infection manifests by pure red-cell aplasia. These patients have a low or absent specific antibodies, persistent or recurrent viremia. Symptoms are fatigue, pallor, and the immune mediated symptoms (rash, arthralgia) are absent. The infection can be a prodrome for another disease and the anemia can remit rapidly after IVIG or antiviral treatment (in HIV patients).

Confirmation of erythroid aplasia secondary to Parvovirus B19 infection can be made through serum antibody anti-B19, PCR – DNA Parvovirus B19 or the pathognomonic appearance of the blood marrow: giant pronormoblasts with large eosinophilic nuclear inclusions and cytoplasmic vacuolization and occasionally "dog-ear" projections may be observed. EM study of the infected cell reveals pseudopod formation, marginated chromatin and virus particles in the nucleus.[5]

There are cases when aplastic anemia can be associated with thrombocytopenia in Parvovirus B19 infections.

Thrombocytopenia related to B19 has two mechanisms: central and peripheral. The central thrombocytopenia is caused by marrow suppression, probably through inhibition of megakaryocyte colony

formation (NS1 protein synthesized by parvovirus). This indicates a parvovirus tropism that exceeds the erythroid series and shows that the viral proteins are toxic even to the cells that do not allow viral replication. The peripheral thrombocytopenia can result after the production of antiplatelets antibodies, and platelet destruction in the reticuloendothelial system.[5]

In our case, parvovirus B19 occurred in an immunossupressed patient, 3 years after kidney transplant. In this case, anemia can have different causes: erythroblastopenia (shown on the bone marrow biopsy), chronic kidney disease related anemia, adverse effect of immunosuppressive drugs, hemophagocytic syndrome, microangiopathic anemia (schistocytes on the peripheral blood smear), intravascular disseminated coagulation (DIC) or sepsis.

Thrombocytopenia is not specific for parvovirus B19 infection, but it can appear. In this case the mechanism is most likely central, by inhibition of megakaryocyte colony formation (bone marrow biopsy: low platelet formation). There are other causes for thrombocytopenia in this case: platelet consumption in DIC, hemophagocytosis and sepsis.

Anemia improved (Hb 7.7 g/dl 10.7 g/dl) and platelets returned to normal after parvovirus infection treatment.

Neurologic manifestations in parvovirus B19 infection:

Parvovirus B19 was associated with various clinical syndromes, including neurological symptoms. The most frequent neurologic manifestation is encephalitis, that has no specific symptoms, with the exception of the rash, arthralgia and anemia, when they occur (usually absent in immunossupressed patients). Cerebrospinal fluid (CSF) analysis is variable and rarely diagnostic. Imaging studies (IRM, CT) show ventricular dilatation, frontal and occipital vasogenic edema), but the majority of studies are normal.[3]

The neurologic findings were: mental status changes, coma, seizures, status epilepticus. The seizures were usually generalized. Localized neurologic symptoms were described: hemiparesis, facial paresis, chorea affecting the face and upper limbs, opsoclonus, diplopia, nistagmus, optic neuritis, paresthesias, apraxia and aphasia. Half of the patient showed improvement, some had sequelae (epilepsy, cognitive defects, spastic quadriplegia) and others died (they had encephalitis and underlying diseses).[4]

Other neurologic findings were: meningitis, stroke, brachial plexus neuropathy, carpal tunel syndrome, acute cerebellar ataxia, Guillain-Barre syndrome involving the legs, velopalatine hemiparesis.[4]

Imaging studies on patient with encephalitis /encephalopathy/meningoencephalitis showed diffuse increased signal in the white matter of the frontal and parietal lobes and enlarged ventricles.[4]

The mean duration of all CNS manifestations in immunocompetent patients was 38 days (2-198 days). In immunossupressed patients the duration was longer, most cases lasting several months.[4]

Our patient presented with encephalitis (memory loss, flapping tremor, dysarthria, seizures and coma). Imaging studies showed enlarged ventricles and also demyelinating lesions in the frontal and parietal lobes and areas of microangiopathic subcortical ischemia. Serial imaging studies in a period of 6 months show steady, non-progressive lesions.

The improvement of the neurologic symptoms after the lowering of the immunossupression therapy and IVIG suggests a neurologic involvement of Parvovirus B19, but the diagnosis can't be certain in the absence of the viral load in cerebrospinal fluid, the antibodies against Parvovirus B19 are not useful in this case.

3 months after the acute episode, the patient has another seizure and is started on antiepileptic therapy.

Hemophagocytic syndrome (HPS)

Virus-associated hemophagocytic syndrome (VAHS) is characterized by histiocytic hyperplasia, marked hemophagocytosis and cytopenia, associated with a systemic viral infection. In contrast to malignant histiocytosis, VAHS is usually benign, self-limited, with reversible histiocytic proliferation. Hemophagocytosis is not uncommon and it occurs in the setting of a wide range of infections. Still, most patients have a degree of immunossupression, usually iatrogenic, so the etiologic role of the infection is sometimes unclear (vs. a coincidental opportunistic infection). There are two reported case of VAHS concurrent with pure red cell aplasia.[5]

Hemophagocytic syndrome was described in renal transplant recipients. More common in the first few weeks after the transplantation, but in few patients it occurred years after transplantation, particularly when it was caused by parasitic infection or neoplasm. Most case were associated with viral infection (CMV, EBV, HH-8, Parvovirus B19, BKV). Other cases were associated with tubesculosis, Escherichia coli, toxoplasmosis, leishmaniasis, etc. Rarely, HPS occurred in patients with T-cell lymphoma or angiosarcoma. However, in a number of cases it was impossible to recognize the cause of HPS.[6]

The diagnosis of posttransplant HPS is not easy, high fever and constitutional symptoms are almost constant, but not specific. Hepatosplenomegaly can be absent in half of the cases. Laboratory investigations generally show pancytopenia, however it can be caused by the immunosuppressive drugs (azathioprine, mycophenolat, etc.) Hypertriglyceridemia is frequent in transplant recipients, particularly when treated with

high doses of steroids, sirolimus or everolimus. High levels of ferritin that are generally associated to HPS, are also increased in other inflammatory conditions. However, serum ferritin levels of >10000 ng/ml associated with high levels of CD163 or CD25 are considered to be reliable markers of macrophage activation and are diagnostic for HPS if there is evidence of hemophagocytosis in the bone marrow. About half of the patients can have neurologic manifestations: stupor, disorientation, seizures and are seen among the most severe cases. The bone marrow can show the characteristic proliferation of mature histiocytes actively ingesting other blood cells. Posttransplant HPS prognosis is more severe than in non-transplant patients.[6]

The treatment of posttransplant HPS is difficult. Efforts should be made to recognize and treat the associated infection. Infusion of intravenous immunoglobulins (IVIg) can be beneficial. What to do with the immunossupression is still an unresolved issue. In most transplant patients with HPS, the immunosuppressive drugs are reduced or withdrawn in order to improve the resistance to infection. On the other hand, cyclosporine and antithymocyte globulins may reduce the activation of Th-1 lymphocytes and the cytokine production and have been recommended in the treatment of HPS in non-transplant patients. A reasonable compromise seems to be to minimize the administration of immunosuppressive drugs while given intravenous high doses steroids. At high doses, steroids can protect from rejection, can reduce the activation of macrophages, the cytokine production and can improve survival in patients with septic shock.[6]

In our case, we raise the suspicion of associated hemophagocytic syndrome, in the context of Parvovirus B19 infection and the following data that support this diagnosis: fever, hypofibrinogenemia, hypertrigliceridemia, hiperferritinemia, medullar hemophagocytosis, bicytopenia, associated viral infection, immunossupressed patient. The neurologic manifestations could be secondary to HPS, as they are described in about half of these patients.

Conclusions:

The article presents the complex case of a renal transplantation patient, which develops anemia followed by severe thrombocytopenia associated with encephalitic neurological manifestations. It is a case of Parvovirus B19 infection in an immunosuppressed patient, complicated with aplastic anemia that responded to lowering the immunosuppression and IVIG.

The particularity of the case is the severe thrombocytopenia and hypofibrinogenemia that persisted even after the infection was resolved, which may be explained by explained by the association of hemophagocytic syndrome.

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