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EXTRAMEDULLARY HEMATOPOIESIS: A CASE STUDY OF NODULAR LOCALIZATION IN THE LIVER

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ABSTRACT
Beta-thalassemia includes a group of monogenic diseases which are characterized by a disturbed synthesis of beta-globin. During the last few years a number of studies showed that pathologic disturbances are genetically determined by an abnormal expression of genes that control the erythropoiesis and iron metabolism. We are presenting a clinical case of a 17-year old man with homozygous beta-thalassemia, who developed extramedullary hematopoiesis with liver localization. In most cases, extramedullary hematopoiesis expresses no clinical symptoms and is discovered accidentally during ultrasound examination and CT scanning. Our aim was to check the status of JAK 2 tyrosine kinase – whether it's gene is normal and whether it's accompanied by mutation that would be the basis for the non-effective erythropoiesis and extramedullary hematopoiesis in the liver of this patient.

Key words: β-thalassemia, extramedullary hematopoiesis, chelator

INTRODUCTION

Beta-thalassemia includes a group of monogenic diseases which are characterized by a *disturbed synthesis of beta-globin* (1, 17). Abnormalities are entirely heterogenic and produce a wide spectrum of phenotypes ranging from patients with almost no symptoms to those who require regular blood transfusions for life support. As a result of the disturbed synthesis of beta-globin patients suffer from chronic anemia caused by a process called *ineffective erythropoiesis* (IEE). It leads to the development of *extramedullary hematopoiesis* (EMH) with splenomegaly and extreme iron overload resulting in many of the secondary pathological processes observed in beta-thalassemia patients. During the past few years a number of studies have shown that such secondary pathological processes are genetically determined by an abnormal expression of genes which take part in controlling the erythropoiesis and iron metabolism. Despite the fact that beta-thalassemia is one of the first monogenic diseases, only recently the scholarly community began focusing its efforts on the real molecular mechanisms that underlie this disease, finding new and more optimistic perspectives for patients suffering from it around the world.

PRESENTING THE CASE

Tumor-like extramedullary hematopoiesis with localization in the liver in patients with beta-thalassemia is a rare clinical phenomenon which causes a number of problems with the differential diagnosis.

We are presenting a clinical case of a patient with homozygous beta-thalassemia, who developed extramedullary hematopoiesis with localization in the liver. The patient is a 17-year old man suffering from homozygous beta-thalassemia caused by IVS-I-110 mutation in the β-gene.

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He was diagnosed with the disease at infant age. Since then, the patient has been given monthly blood transfusions, and since the age of four, he has been receiving chelation therapy with Desferal. Since October 2010, Ferriprox has been used for the therapy.

The man is living at the Children's Home "Maria Theresia" in the town of Stara Zagora. He has physical developmental delay, but appropriate neuropsychological development for his age.

Past diseases:

- frequent upper respiratory tract infections, bronchitis and pneumonia
- splenectomized in September 2005.

Family anamnesis is incomplete for obvious reasons (the kid was abandoned by his parents).

Status: Admitted in compromised general condition, afebrile, with slightly expressed thalassemic facies. The skin and the visible mucous membranes were pale, icteric. Adipose tissue under the skin was moderately present. Heart condition was rhythmic with the rate of 96 per min. Protomezosystolic murmur, level 2/6 at the ERB was determined. Arterial pressure – 110/60 mmHg. The abdomen was soft, not painful, with cicatrix from previous splenectomy. The liver was palpated 4 sm above the navel horizontal (6 sm under the thorax) with solid-elastic consistency and tapered edge. The ophthalmologic examination didn't show any pathological deviations.

Paraclinical examinations: The blood tests showed accelerated erythrocyte sedimentation rate (ESR) of 34 mm;
Hb 77 g/l;
Er 2.63 x 10^{12}/l;
Thr 565 x 10^{9}/l;
Leuc 25.3 x 10^{9}/l;
DBP: Gran 37.6 %; Ly 55.1 %; Mid 7.3 %;
Erythroblast in the **peripheral blood** 85/100;
Ret. 41 ‰;
Normal differential blood picture;
Blood sugar 5.69 mmol/l;
Urea 3.65 mmol/l;
Creatinin 31 μmol/l.

The iron in the serum is 40 μmol/l, ICC (iron connecting capacity) 10.3 μmol/l; Ferritin – 2500 ng/ml.

LDH 139; total bilirubin 33.3 μmol/l; direct fraction – 12.7 μmol/l.

Coagulation status – not changed. Alkaline phosphatase, ASAT 22.1 U/l; ALAT 29.9 U/l, GGTP, Serum cholinesterase, total protein and albumin и албумин are normal.

Coombs test – direct (+) plus, indirect (-) minus; aloerythrocyte antibodies and autoerythrocyte antibodies (-) minus.

Urine – slightly increased urobilinogen.

Serology for toxoplasmosis – (-) munus.

Serology for echinococcosis – (-) munus. – ELISA 0.87.

Tumor markers – (-) munus. (CEA – 0.587 ng/ml; AFP – 1.38 IU/ml; CA – 19.9 – 4.75 U/ml).

Hepatitis markers: HBsAg (-) munus.; Anti HCV (-) munus.

Y.enterocolitica antibodies: gr.03 under 1:50 (-) minus; gr.0:9 under 1:50 (-) minus (norm ≤ 1:50).

The results of HPLC are presented in **Fig. 1.**

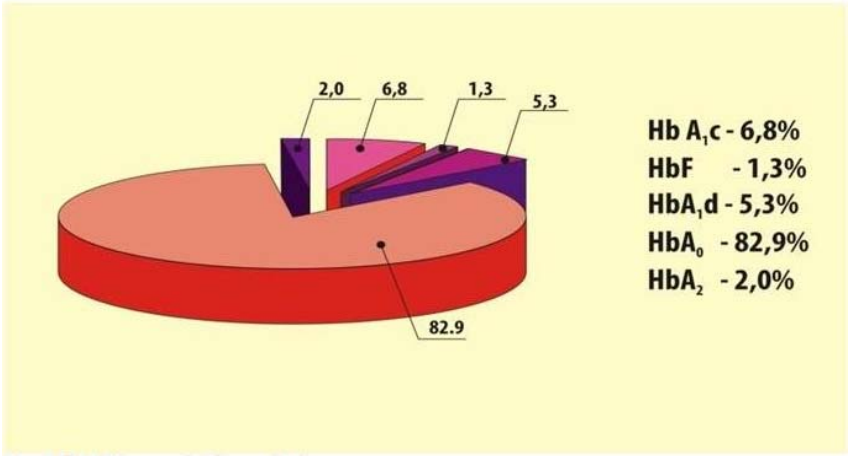

Fig. 1 HPLC hemoglobin analysis

The accession of the iron status determined via T_2*MRI on October 11, 2011 is presented in **Table 1.**

Table 1
Values of the left ventricle parameters

Telediastolic volume (mL)	Telesystolic volume (mL)	Stroke volume (mL)	Ejection fraction (%)	LV mass (g)
142	45	97	68	61

Organ	T2*	Level of deposition
Heart	16.39 ms	Low
Liver	1.50 ms	Moderate

Conclusion:

1. Low level of iron overload in the heart muscle.
2. Moderate level of iron overload in the liver.
3. Value of the ejection fraction of the left ventricle ~68%

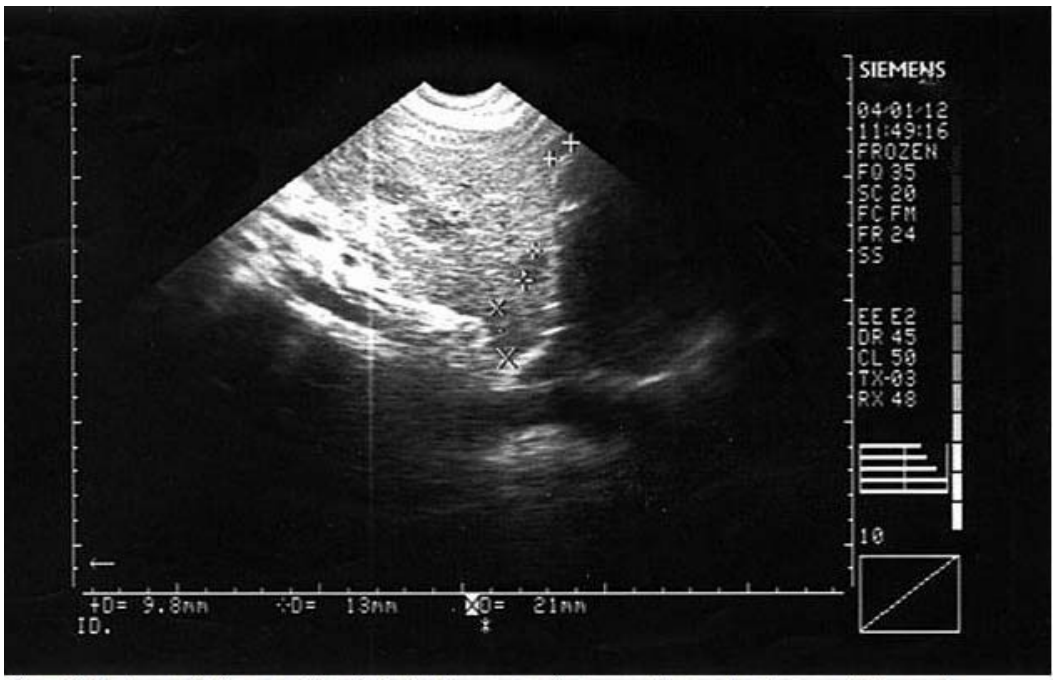

Fig. 2. Enlarged liver with slightly increased parenchymal echogenicity and a considerable number of hypoechogenic zones of different sizes (9.8 to 23 mm)

The abdominal echography performed on January 1, 2012 **(Fig. 2)** showed evidence of enlarged liver at the right midclavicular line (132 mm) with slightly increased parenchymal echogenicity. In both liver lobes and all segments, many rounded hypoechogenic zones of different sizes (9.8 to 23 mm) could be seen.

Gallbladder – normal size, without thickened walls, no concrements.

Pancreas – normal size and structure.

Spleen – n/a (surgically removed at the age of 7).

Kidneys and suprarenal glands – normal topography and sizes, preserved normoechogenic parenchyma; pyelo-calix system – no deviation.

In segments 2 and 3 of the left liver lobe, other hypoechogenic focuses were seen, diameter of 17 and 20 mm **(Fig. 3).**

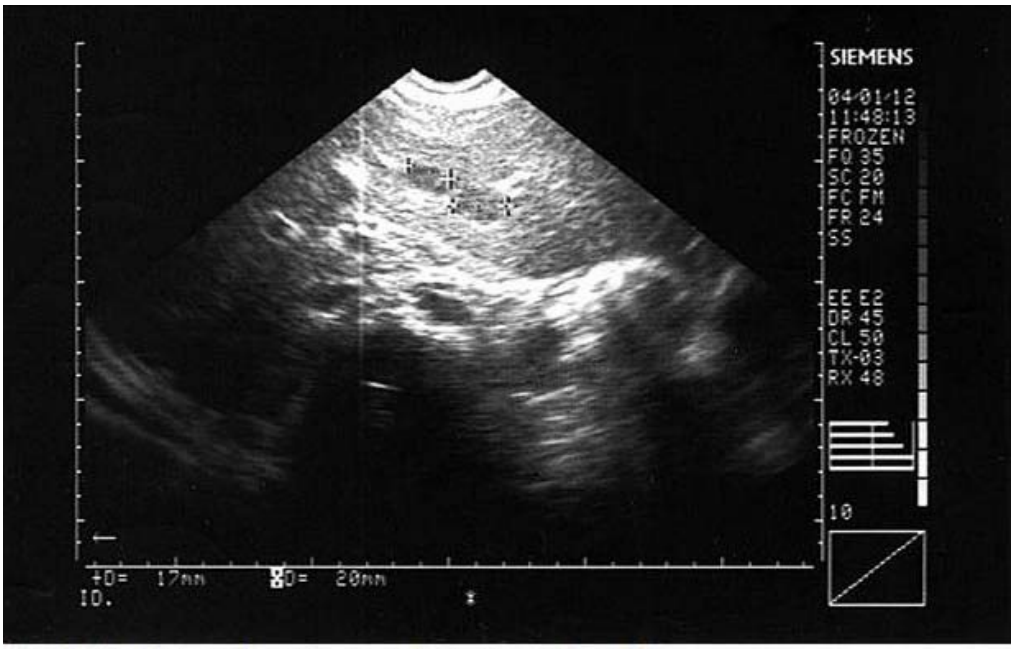

Fig. 3. Two hypoechogenic zones in segments 2 and 3 of the left liver lobe; diameter of 17 and 20 mm

On January 15, 2012, through Doppler echography a significant dynamics of the number and size of hypoechogenic focuses was established. The test showed several formations with size of 10–12 mm, highly blood-supplied **(Fig. 4).**

The above-described hypoechogenic focuses didn't affect the normal architectonics of the liver vessel system **(Fig. 5)**.

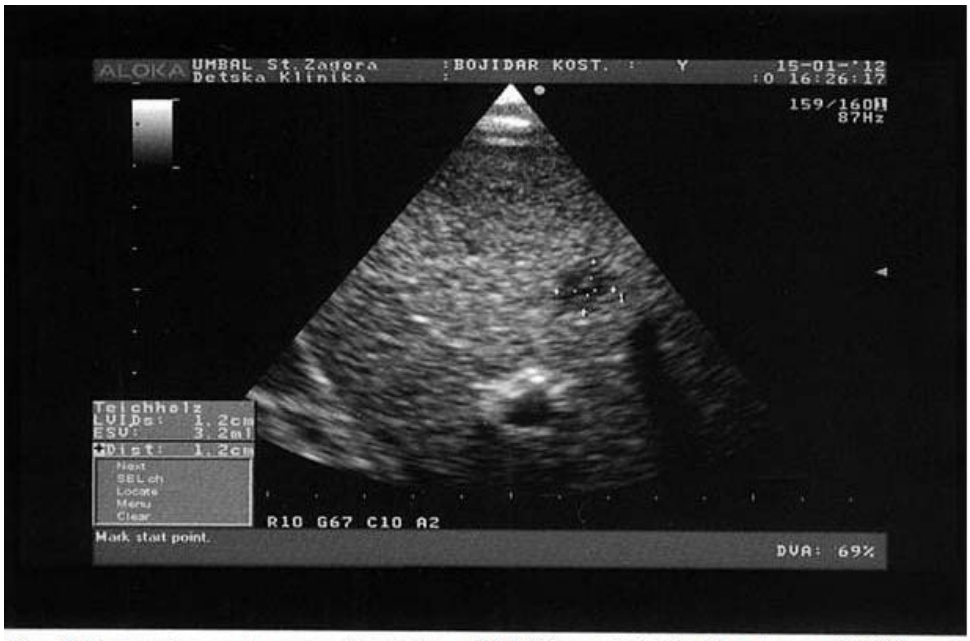

Fig. 4 Hypoechogenic zones, diameter of 10-12 mm, highly blood-supplied (Doppler echography)

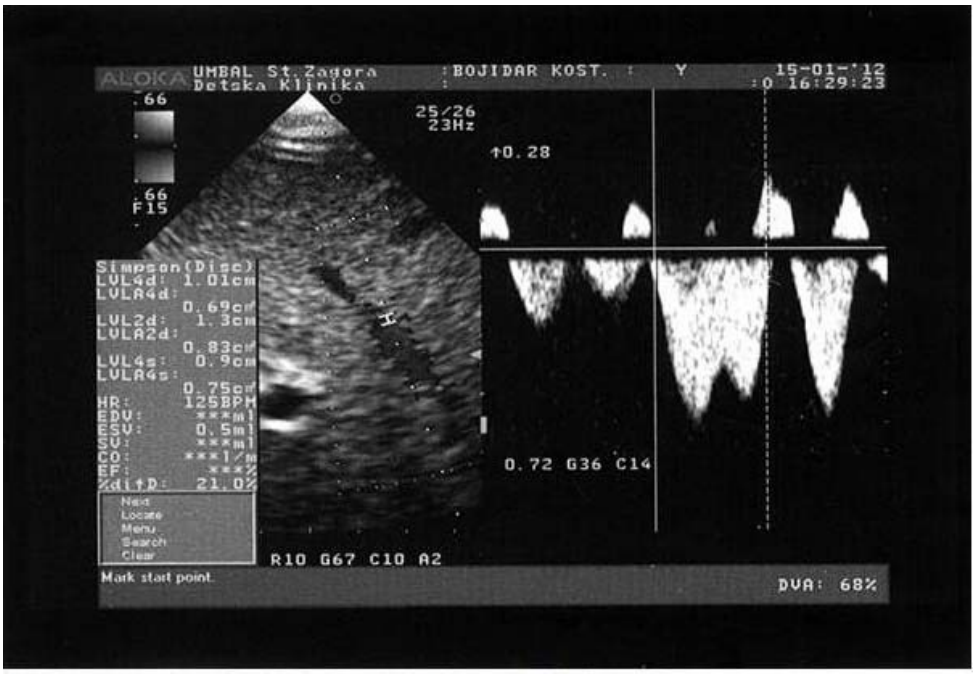

Fig. 5. Normal architectonics of the liver vessel system

On February 3, 2012 it was determined that the reversed development of the initially discovered changes in the parenchyma of the liver was still in progress: the liver at the right midclavicular line was 117 mm in size and the number of the hypoechogenic focuses decreased to 2 – one with diameter of 14 mm in segment 4 the other with diameter of 10 mm in segment 6.

On February 20, no nodular changes of parenchyma of the liver were determined. The control echography test performed on March 22, 2012 showed no more focuses in the liver **(Fig. 6).**

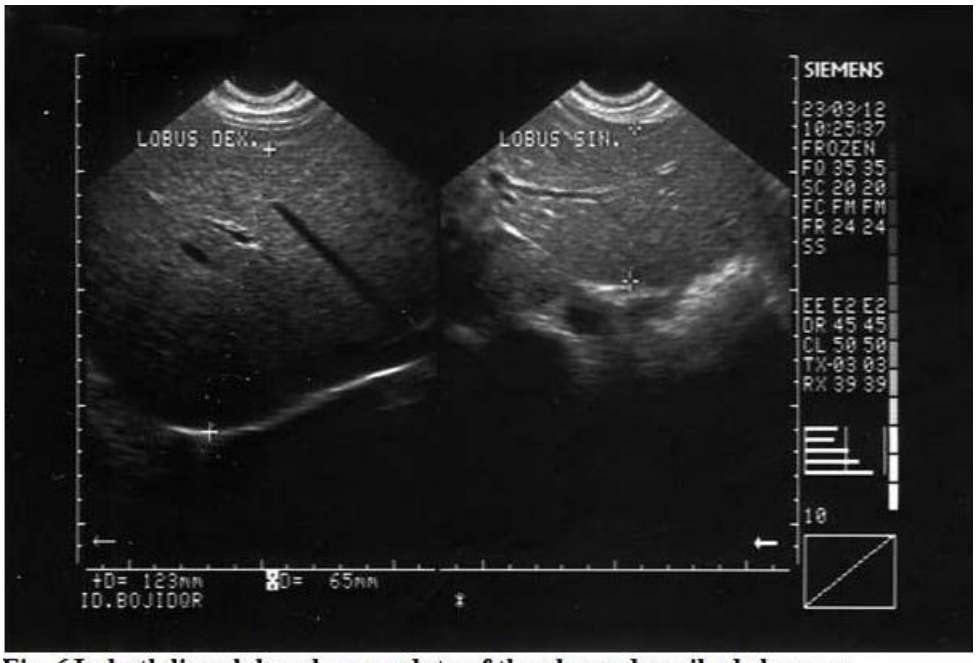

Fig. 6 In both liver lobes show no data of the above-described changes

Treatment:
Three blood transfusions of isogroup and no leukocyte erythrocyte concentrate 250 ml i.v., chelation therapy with Desferal and Exjade, Medaxon, Amikacin, Carsil.

The condition gradually improved, and the changes in the liver reversed.

Discussing the case:
Tumor-like extramedullary hematopoiesis develops as a result of compensatory

hyperplasia in patients with chronic inherited anemia like thalassemia and chemoglobinosis, myelodoplastic and myeloproliferative syndromes, secondary myelofibrosis, etc. (5).

In most cases extramedullary hematopoiesis is found in thorax: bone vertebral angle, retro-mediastinum and parenchyma of the liver (14, 19).

Next in frequency is intra-abdominal extramedullary hematopoiesis (EMH) – in the paraspinal zone, retro-peritoneum, next to the kidneys, in the spleen and liver (16, 6). The sizes of EMH vary – from 1.5 to 15 sm. Usually EMH have no symptoms. However, the various studies have described mediastinal syndromes, syndromes with bone marrow compression, renal dysfunction and other clinical manifestations (19, 7).

In the cases with progressive increase of EMH zones local radiotherapy, chemotherapy with Hydrea and surgical resection where necessary is used.

EMH localization i nthe liver is very rare (2). Only a few cases of EMH have been reported in patients with beta-thalassemia (18). Symptoms depend on the size of the formation and its localization in the liver, the possibilities for compression of the billiar tract and the big blood vessels.

Most often extramedullar formation in the liver has no symptoms and is discovered accidentally during ultrasound examination and CT scanning. In most cases, the discoveries have been initially interpreted as liver carcinoma, metastatic tumors, parasitic cysts, etc.

Changes in the liver of our patient were also worrisome. We had to eliminate primary liver carcinoma or other malign process that was the cause for metastases in the liver.

The examinations eliminated the liver carcinoma as well as metastatic focuses of extrahepatic malign processes. Besides the routine blood markers, the JAK-2 gene was examined to determine if there was V617F mutation present.

What is JAK2 – a gene that controls the production of erythrocytes **(Fig. 7)**.

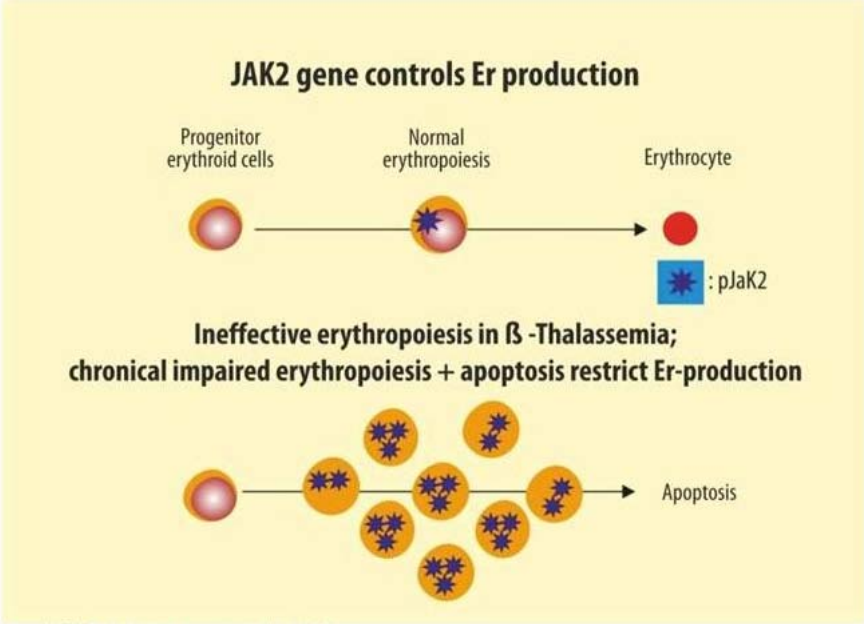

Fig. 7 (Melchiori L., et. al.) (12)

It's a known fact that in cases with beta-thalassemia the erythropoietic process is seriously disturbed and is called process of *ineffective erythropoiesis.*

Hypoxia resulting from the permanent loss of damaged erythrocytes induces extreme increase of the levels of erythropoietin (EPO) in patients with β-thalassemia, causing bone marrow hyperplasia and bone deformations.

This traditional point of view was the reason for focusing on the apoptotic aspect of the ineffective erythropoiesis (4, 15), which is important, but not the only possible one.

Recent studies have shown that along with apoptosis a significant number of erythroid progenitors undergo increased proliferation and decreased differentiation (11).

It was determined in an experimental model that the high level of EPO increases surviving and proliferation of the erythroid progenitors despite the fact that there is no efficient differentiation and normal erythrocytes formation (13).

It was determined that this process is connected with phosphorylited forms of JAK2 kinase that lead to a higher number of proliferating thalassemic erythroid progenitors than normally proceeding processes **(Fig. 8)**.

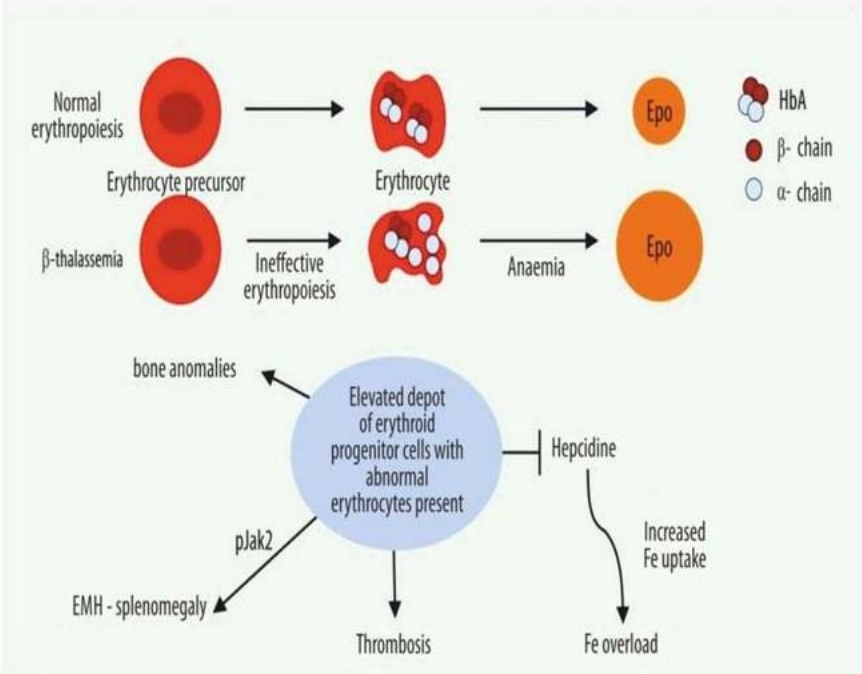

Fig. 8. Scheme of erythropoiesis normal and in cases of β-thalassemia (Gardenghi S., et. al.) (5)

It looks like JAK2 V617F mutation causes cytokine-independent activation of the paths of transmitting a signal by the erythropoietic cells.

JAK2 is a member of JAK tyrosine kinase family and mediates the operation of many other cytokines besides EPO.

What makes the combination between EPO and JAK2 unique? It's the fact that JAK2 is the only kynase that is connected with EPOR and thus the only signal transductor of EPO.

In 2005, five research teams – Baxster, E.I. et al 2005 (3); James et al 2005 (8); Kralovics et al 2005 (9); Levine, R. et al 2005 (10); Zhao, R et al 2005 (20) –independently described an activating mutation (V617F) in the pseudokinase domain of JAK2 which probably causes cytokine-independent activaton of the paths for transmitting signals of erythropoietic receptors.

All these studies showed that hyperactivation of JAK2 causes massive erythropoietic activity and huge expansion of erythrone with respective splenomagalia – a phenotype which is identical to that in β-thalassemia.

It was determined that this process is related to the phosphorylated form of JAK2 kinase which results in a higher number of proliferating thalassemic erythroid progenitors in comparison with normal erythropoiesis (see **Fig. 8**).

The persisting phosphorlation of JAK2 as a result of the high levels of EPO induces massive extramedullary hematopoiesis with early erythroid progenitor colonization followed by proliferation mainly in the spleen and liver (12).

Considering these processes underlying the ineffective erythropoiesis and extramedullar hematopoiesis as connected with the JAK2 tyrosine kinase, we set the objective to check the status of JAK2 kinase and determine whether its gene is normal and if it's combined with V617F-mutation.

The tests shown in **Fig. 9** didn't determine deviations in the gene for JAK2 kinase. In addition, no V617F-mutation was found.

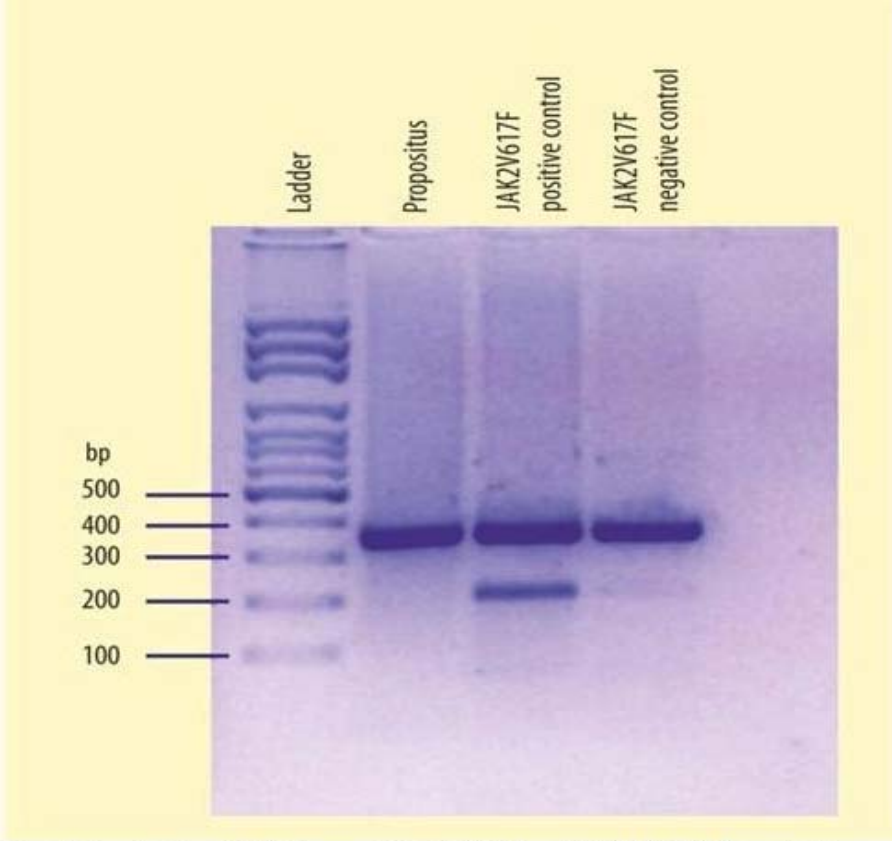

Fig. 9 Analysis of allele specific PCR for JAK2V617F mutation through electrophoresis of 1.5% agarose gel

Most likely this is the cause for the relatively light form of the extramedullar hematopoiesis in the liver of our patient.

After the adequate blood-transfusion and helation therapy the condition of the patient improved and the changes in the liver reversed.

Apparently JAK2 kinase is an important factor for the normal hematopoiesis and the research in the area will discover possibilities for new therapeutic strategies.

REFERENCES

1. Ефремов Г. Д., Петков Г., Чакърова, П. Таласемични синдроми. 2003, КОТА ООД Стара Загора.
2. Aytac S., Fitoz, S., Akyar S. et al. Focal intrahepatic extramedullary hematopoiesis: color Dopler and CT findings. *Abd. Imaging.* 1999; 24:366-368.
3. Baxter E.J., Scott L.M., Camphell P.J. et al. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. *Lancet* 2005; 365:1054-1061.
4. Centis F., Tabellini L., Lucarelli G. et al. The importance of erythrocid expansion in determining the extent of apoptosis in erythroid precursors in patients with beta-thalassemia major. *Blood* 2000; 96(10):3624-3629.
5. Gardenghi S., Robert W.G., Rivella S. – Anemia, Ineffective Erythropoiesis and Hepcidin: Interacting Factors in Abnormal Iron Metabolism Leading to Iron Overload in β-Thalassemia. Hematol. *Oncol. Clin. North Am.* 2010 December; 24 (6):1089-1107.
6. Gemenis T., Philippou A., Gouliamos A., et al. Atypical localisation of extramedullary hemopoietic masses in thalassemia. *Radiologe*, 1989; 29:295-296.

7. Jackson, D.V. Jr., Randall, M.E., Richards, F. Spinal cord compression due to extramedullary hematopoisis in thalassemia : long term follow – up after radiotherapy. *Surg. Neurol.* 1998; 29:389-392.
8. James C, Ugo V, Le Couedil J.P. et al. A unique clonal JAK2 mutation leading to constituitive causes polycythemia vera. *Nature* 2005; 434:1144-1148.
9. Kralovics R, Passamonti F, Buser A.S., et al. A gain-of-functions mutation of JAK2 is myeloproliferative disorders. *N. Eng. J.Med.* 2005; 352:1779-1790.
10. Levine R.L., Wedleigh M, Cools J., et al. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia and myeloid metaplasia with myelofibrosis. *Cancer Cell.* 2005; 7:387-397.
11. Libani I.V. Guy E.C., Melchiori L., et al. Decreassed differentiation of erythroid olls exacerbates ineffective erythropoiesis in beta thalassemia. *Blood* 2008; 112(3):875-885.
12. Melchiori, L., Gardenghi, S., Rivella, S., Beta Thalassemia: HiJAKing ineffective Erythropoiesis and Iron Overload. *Advances in Hematology,* Vol 2010;2010:938640.
13. Rivella. S. "ineffective erythropoiesis and thalassemias" *Curr Opin Hematol,* vol.16, no.3, 187-194, 2009.
14. Sakai S., Okushima K., Yamashita N., Shimoda K. A case of extramedullary hematopoiesis in thalassemia. *Rinsho Hoshasen*, 1990, 35:879-881.
15. Schier S.L, Pathophysiology of thalassemia. *Curr. Opin. Hematol*. 2002; 9:123-126.
16. Vlahos L., Trakadas S., Gouliamos A., et al, Retrocrural masses of extramedullary hematopoiesis in β-thalassemia. *Magn. Reson. Imaging.* 1993; 11:1227-1229.
17. Weatherall D.J., and Clegg, J.B. The Thalassemia syndromes, 4th ed Oxford Blackwell Scientific Pubs, 2001.
18. Wong Y., Chen F., Tai K.S. et al. Imaging features of focal intrahepatic extramedullary haematopoiesis. *Br. J. Radiol.* 1999; 72:906-910.
19. Yu Y.c, Kao E.L., Chou S.H et al. Intrathoracic extramedullary hematopoiesis simulating posterior mediastinal mass-report of a case in patient withβ-thal. intermedia. Kao Hsiung, I. Hsueh, Ko Hsueh Tsa Clin. 1991; 7:43-48.
20. Zhao R, Xing S, et al. Identification of an acquired JAK2 mutation in polycythemia vera. *Journal of Biol. Chem*. 2005; 280:22788-22792.