Short communication

Potential Causes of Anemia in Hepatic Cirrhosis

Răzvan Jugănaru[2], Mara Alexandra Moldovan[2], Ana-Maria Deleanu[1]

[1]Spitalul Clinic „Theodor Burghele", Bucureşti, România
[2]Spitalul Universitar de Urgenţă Bucureşti, România

ORCID: 0009-0002-3441-3199 - https://orcid.org/0009-0002-3441-3199

Correspondence to: Răzvan Jugănaru, e-mail: razvan.juganaru@rez.umfcd.ro, razvan.juganaru42@gmail.com

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List of abbreviations: aa – arteries, **(AI)HA** - (Autoimmune)Hemolytic Anemia, **Ab** - Antibodies, **ADAMTS13** - A Disintegrin And Metalloproteinase with a ThromboSpondin type 1 repeats-member 13, **AT-III** - AntiThrombin-III, **APLS** - antiphosphlipid syndrome, **ATN** – Acute Tubular Necrosis, **BC** - blood count, **BMA** - Bone Marrow Aspiration, **BP –** blood pressure, **Cl** - clearance, **CPP** - Cryoprecipitate, **CVA** - Cerebral Vascular Accident, **δ-ALA** Dehydratase - Acid Delta Aminolevulinic Dehydratase, **δ-ALAS** - Acid Delta Aminolevulinic Synthase, **DB** – direct bilirubin, **DCT** - Direct Coombs Test, **D** - day, **DD** – D dimers, **DIC** - Disseminated Intravascular Coagulation, **DVT** - Deep Venous Thrombosis, **EC(B)V** - Effective Circulating (Blood) Volume, **EPO** - erythropoietin, **FDPs** - Fibrinogen (Fibrin) Degradation Products, **FFP** - Fresh Frozen Plasma, **G6PD** - glucose-6-phosphate dehydrogenase, **GI** - Gastrointestinal, **HP** - histopathological, **HRS** - hepatorenal syndrome, **Ht** – hematocrit, **HVB** – Hepatitis Virus B, **HVC** – Hepatitis Virus C, **ICA(U)** - Intensive Care Anesthesia (Unit), **IB** – indirect bilirubin, **ICT** - Indirect Coombs test, **IL-**6 - interleukin 6, **im** - intramuscular ,

iv - intravenous, **ivi** - intravenous infusion, **LAT** - Lysophosphocholine-Acyl-Transferase, **LCVE** - Liver Cirrhosis of Viral etiology, **LDH** - Lactate Dehydrogenase, **LMWH** - Low Molecular Weight Heparin, **MAO** - MonoAminoOxidase, **MDS** - MyeloDysplastic Syndrome, **MM** - Multiple Myeloma, **NO** - Nitric Oxide, **NSAID** - Nonsteroidal anti-inflammatory drugs, **PBS** - Peripheral Blood Smear, **PGAA** - monooxygenase - Peptidyl Glycine Alpha-Amidating-monoxygenase, **po** - per os, **PoHT** - Portal Hypertension, **PK** – pyruvate kinase, **PNH** - Paroxysmal Nocturnal Hemoglobinuria, **PPIs** - Proton Pump Inhibitors, **PRBC** - Packed Red Blood Cells, **RF** - Rheumatoid Factor, **RI** - Renal Insufficiency, **S** - week, **sc** - subcutaneous, **SOD** - SuperOxide Dismutase, **TA(s)** - blood pressure (systolic), **TG** - Triglycerides, **TIA** – Transient Ischemic Accident, **TIBC** - Total Iron Bindig Capacity (the total iron binding capacity to transferrin), **TIPS** - Transjugular Intrahepatic Portosystemic Shunt, **TSC** - Transferrin Saturation Coefficient, **TTP** - Thrombotic Thrombocytopenic Purpura, **v. / vit.** - vitamin, **VLC** - viral liver cirrhosis, **VWF** - Von Willebrand Factor, **W** – week, **WM** - Waldenström Macroglobulinemia.

Abstract

The article looks at the basic mechanisms of anemia in patients with liver cirrhosis (bleeding through esophageal varices, hemorrhoids, vascular fragility, hypertensive portal gastropathy, thrombocytopenia, coagulopathy, vitamin K deficiency, splenic rupture, splenic sequestration of red blood cells in splenomegaly) and also the additional mechanisms of anemia in liver cirrhosis (erythropoietin deficiency, renal injury, chronic inflammation, deficiency of trace elements and vitamins, autoimmune hemolytic anemia, microangiopathic hemolytic anemia, Zieve syndrome). Depending on the Child-Pugh staging of liver cirrhosis, as well as the associated pathologies of the patient with liver cirrhosis, it is possible that there are simultaneously several ways of the appearance of anemia. It is an attempt to achieve more than a simple review of them in a tabular form, focusing not only on physiopathology, but also on diagnostic and therapeutic elements - the latter being briefly mentioned in widely known situations and presented in more detail in the context of the rarer causes of anemia in patients with liver cirrhosis. In conclusion, the differential and positive diagnosis of anemia in liver cirrhosis decisively influences the therapeutic decision and, of course, not only survival, but also the patient's quality of life. Interdisciplinary collaboration (family doctor, gastroenterologist, internist, infectious disease specialist, hematologist, surgeon, ICA, medical imaging, laboratory) is the optimal way to a diagnosis and targeted treatment of the causes of anemia in liver cirrhosis.

Potential causes of anemia from cirrhosis

1. Bleeding from esophageal varices, internal hemorrhoids, external hemorrhoids

The patient has collateral circulation established on the background of portal hypertension. BC with hypochromic, microcytic anemia. Perform sideremic profile (Fe, ferritin, TSC, TIBC). Treatment of bleeding through esophageal varices:

LINE I: banding with rubber rings, sclerotherapy with absolute alcohol or, if no results are obtained by these methods, TIPS.[1]

LINE II: Sengstaken Blakemore probe. To these are added: PRBC transfusion, systemic hemostatic treatment, BP support, treatment of the underlying disease.

Prophylactic treatment: propranolol, nitrates (when beta-blockers are contraindicated). Treatment of hemorrhoidal bleeding in patients with cirrhosis and portal hypertension:[2]

1. First of all, it is necessary to correct the coagulopathy from cirrhosis, as well as to ensure the treatment of PoHT (TIPS, propranolol).

2. Ligation by banding with rubber rings of hemorrhoidal packs is contraindicated due to the high risk of postprocedural bleeding; in this situation, sclerotherapy is a safe and effective procedure; in cases refractory to sclerotherapy, ligation with suture, hemorrhoidectomy can be performed.[1]

3. Local treatment of hemorrhoids: vasoconstrictor, cicatrizing, painkillers.

2. Bleeding due to vascular fragility

In cirrhosis, glycogen reserves being reduced, lipids and also proteins are used in gluconeogenesis.[3] The reduction of protein synthesis, on the background of the acceleration of protein catabolism, damages the resistance of the vascular wall, collagen being the basic protein from the structure of the vascular wall.[4] BC with hypochromic, microcytic anemia. Perform sideremic profile (Fe, ferritin, TSC, TIBC).

3. Bleeding of the gastric mucosa

Edema of the gastric mucosa in hypertensive portal gastropathy, decreased mucus secretion, increased HCl secretion can lead to diffuse or localized bleeding of the mucosa - erosive gastritis, gastric ulcer. BC with microcytic hypochromic anemia. Perform sideremic profile (Fe, ferritin, TSC, TIBC). Therapeutic balancing of cirrhosis, gastric protector (sucralfate, bismuth subcitrate), PPIs are required. The excessive use of PPIs increases the risk of bacterial overgrowth in the digestive tract leading to the occurrence of spontaneous bacterial peritonitis![5]

4. Bleeding episodes caused by thrombocytopenia and platelet disfunction (thrombocytopathy)

Thrombocytopenia in cirrhosis can occur through multiple mechanisms:

1. splenic sequestration.
2. hepatic deficiency of thrombopoietin secretion (TPO) - physiologically, the main manufacturer of thrombopoietin is the liver.
3. Platelets sequestered in the spleen degrade TPO.[6]
4. Decreased expression of TPO receptors (c-Mpl).[6]
5. Direct medullary suppression of platelet production: alcohol, HVB, HVC, (co)infections with other bone marrow suppressive viral strains - CMV, EBV, Parvovirus B19, HIV.
6. Deficiency of vitamin B_9 (folic acid), vitamin B_{12}. BC with microcytic/normocytic hypochromic anemia. Perform sideremic profile (Fe, ferritin, TSC, TIBC), vitamin B_{12} and folic acid dosage.

Treatment: administration of platelet mass to maintain platelets above 20.000/mmc or above the limit from which bleeding occurred, hemostatic treatment, treatment of the underlying condition. TPO analogs such as Avatrombopag or Lusotrombopag can be administered - Eltrombopag, a first-generation analog of TPO, which is associated with a high risk of thrombotic complications of the portal vein, but also arterial, is prohibited.[1]

If the patient has to perform invasive interventions, the following aspects must be taken into account:[1]

1. In order to have enough time to increase the number of platelets, TPO analogues will be administered at least 3-5 days before the intervention.
2. Platelets > 50.000/mmc.
3. Ht > 25%;
4. Fibrinogen > 120 mg/dl.

Thrombocytopathy induced by: alcohol, FDPs, uremia.[6]

5. Bleeding on the background of coagulopathy from cirrhosis

1. The liver is an important organ in the synthesis of coagulation factors (I - fibrinogen, II - prothrombin, V, VII, IX, X, XI, XII, XIII, prekallikrein - Fletcher factor, HMWK - Fitzgerald factor). In liver cirrhosis, there is a deficiency in the synthesis of these coagulation factors, a deficiency that correlates with the level of hypoalbuminemia. Insufficient concentrations of the coagulation factors listed above can contribute to the occurrence of hemorrhages, with the exception of the last three factors mentioned, whose deficiency in hepatic cirrhosis is not correlated with the hemorrhagic diathesis.[6]
2. Coagulopathy can also occur in conditions when fibrinogen has a normal serum concentration, FDPs (for instance: DD) are within normal limits, but the fibrinogen molecule shows alterations of a qualitative nature (dysfibrionogenemia). This phenomenon leads to the appearance of some fibrin monomers that polymerize defectively, moreover the qualitatively abnormal fibrinogen has activity similar to AT-III, preventing clot formation.[6]
3. Endogenic activators of plasminogen have a reduced hepatic clearance in cirrhosis, persisting longer in the circulation and activating fibrinolysis, facilitating hemorrhagic diathesis. The excess occurrence of FDPs further affects the coagulation and also platelet's function.[6]
4. The loss of coagulation factors in the ascites fluid potentiates the hemorrhagic diathesis.[6] In the case of cirrhotic patients, the risk of bleeding correlates best with the following three biological aspects: platelets below 30.000 / mmc, fibrinogen < 60 mg/dl, APTT > 100 s.[1] BC with hypochromic, microcytic anemia. Perform sideremic profile (Fe, ferritin, TSC, TIBC). Hemostatic treatment: ivi CPP or FFP, ivi hemostatics every 8 hours (adrenostazin, etamsylate, tranexamic acid, phytomenadione), until the end of the hemorrhagic syndrome and the standardization of the coagulation. Administration of vitamin K may have only minimal effects if the liver damage is severe - a situation in which the liver cannot synthesize clotting factors effectively anyway. Recombinant factor VIIa can be administered (increased costs and risk of up to 2% of thrombotic complications).[1]

6. Bleeding due to vitamin K deficiency

Vitamin K (phytomenadione) is important in the synthesis of coagulation factors II, VII, IX, X.

1. Broad-spectrum antibiotic treatments used for the infectious complications of cirrhosis can affect

the bacterial flora of the large intestine, which is involved in the synthesis of vitamin K.
2. Malabsorption of vitamin K, a fat-soluble vitamin, due to pancreatic enzyme deficiency (toxic nutritional pancreatitis and cirrhosis) and/or due to a poor choleretic function of the liver. Deficient lipid emulsification and digestion causes steatorrhea, fat-soluble vitamins (A, D, K, E, F) being lost in this way.
3. Malabsorption of vitamin K due to edema of the colonic mucosa, caused by hypoalbuminemia and portal hypertension.
4. Vitamin K intake deficit. BC with hypochromic, microcytic anemia. Perform sideremic profile (Fe, ferritin, TSC, TIBC).

Treatment: CPP or FFP ivi, hemostatics ivi every 8 h (adrenostazin, etamsylate, tranexamic acid), administration of vitamin K 1 f ivi/at 6-8 h/day, until the end of the hemorrhagic syndrome and the normalization of the coagulation. Caution: Etamsilate can lower BP and cause bronchospasm! Tranexamic acid (Ugurol, Exacyl) is administered depending on creatinine Cl and only after performing urinalysis beforehand to rule out the presence of red blood cells in the urine! Tranexamic acid can cause hematuria and mechanical anuria through the formation of ureteral clots. Tranexamic acid is contraindicated in severe RI (risk of accumulation). As already mentioned, the administration of vitamin K can only have minimal effects if the liver damage is severe - see point 5.

7. ECV redistribution in case of splenomegaly

Part of the figurative elements of the blood are constantly transiently captive in an enlarged spleen.[7] Treatment of cirrhosis, treatment of PoHT.

8. Splenic rupture

Splenic rupture determines massive internal bleeding in one or two steps. It leads to acute blood loss requiring emergency hydroelectrolytic, hematological rebalancing and surgical treatment - splenectomy. Following splenectomy, must be ensured the management of:
1. Venous thromboembolism in the first 24 hours postoperatively (administration of sc LMWH - enoxaparin - if there are no contraindications);
2. Post-interventional thrombocytosis, which can reach > 1.000.000/mmc and usually occurs in weeks 1-3 after the intervention (75-80% of patients),[8] happens through multiple mechanisms:

a) postoperative inflammatory syndrome +/- infectious syndrome which, by cytokines, stimulates the proliferation and maturation of megakaryocytes and an accelerated release of platelets from the marrow;

b) blood loss, leading to the stimulation of marrow progenitors that are common to both lineages - erythrocyte, megakaryocyte;

c) lack of splenic reservoir leads to decreased platelet sequestration.[9]

Thrombocytosis alone is not sufficient to lead to a clinically significant thrombosis. Aspirin (75 mg/day po) can only be useful if the platelets exceed 1 million/mmc or in patients with thrombocytosis and additional thrombogenic risk factors (cases in which additional targeted treatment had already been administered – patients with thrombosis, thrombophilia, hyperhomocysteinemia, liver diseases, atherosclerosis, dyslipidemia, obesity, smokers, diabetics, neoplasms, hyperviscosity syndromes, patients with APLS, HIV, inflammatory bowel diseases, hypo- or hyper-thyroidism and so forth);[8]
3. Perioperative antibiotic prophylaxis (iv cephalosporins);
4. Prophylaxis of infections with encapsulated germs (Streptococcus pneumoniae, Neisseria meningitis, Hemophilus influenzae): pneumococcal vaccination 2 weeks after emergency surgery and a booster 5 years later if the antibody titer is low; Haemophilus Influenzae type b and group C meningococcal vaccination which it is done simultaneously with the pneumococcal vaccination 2 weeks after emergency surgery (Menitorix - Hib/Men C vaccine) and a booster 2 months later – the booster should be done only in the case of those patients who were not previously vaccinated with Menitorix according to the national program;[10]
5. Antibiotic prophylaxis should be done at least 2 years post-splenectomy: penicillin V 500 mg po every 12 h (in case of reduced compliance only 500 mg po/24 h can be administered), ampicillin 250-500 mg po/day.[10] In case of allergy or reduced compliance at beta-lactams, macrolides can be administered: erythromycin or clarithromycin (preferably, clarithromycin being better tolerated due to a lower profile of digestive adverse reactions), 250-500 mg po/day. Macrolides also have the advantage of a minimal effect on the intestinal commensal flora (diarrhea after the administration of macrolides usually occurs by accelerating gastric emptying, a markedly reduced effect in the case of clarithromycin), as well as an immunomodulatory effect compared to beta-lactams.

9. Bone marrow suppression

Depending on the etiology of cirrhosis, direct bone marrow suppression of erythrocyte precursors can occur either because of viral strains (HVC, HVB) or because of alcohol intake, with the same result: anemia. In patients with alcoholic liver damage, including those with toxic nutritional liver cirrhosis, secondary acquired anemia with ringed sideroblasts can occur.[11] Alcohol itself does not increase the risk of myelodysplastic syndrome[12] but chronic inflammation, which can be associated with alcoholism, lack of vitamin B_6 (fundamental in heme synthesis[13] - the key coenzyme of δ-ALAS) are associated with reduced activity of δ-ALAS, inhibition of δ-ALA Dehydratase (Porphobilinogen Synthetase) by alcohol or in patients who also have diabetes[14] ineffective activity of δ-ALA Dehydratase (Porphobilinogen Synthetase) and Ferrochelatase (Hemsynthetase) in the absence of the essential trace element for their activity, Zn,[14] on the background of dietary Zn deficiency in chronic alcoholism, can simultaneously increase the storage, as well as decrease the use of iron at the level of erythrocyte precursors. In the case of alcohols (brandy etc) manufactured in individual households by distillation in improvised copper boilers, in the manufacture of which Pb was also illegally used, 3 enzymes involved in heme synthesis will be inhibited by Pb[14] (δ-ALA Dehydratase - Porphobilinogen Synthetase, Coproporphyrinogen Oxidase, Ferrochelatase - Hemsynthetase), appearing an additional mechanism by which Fe cannot be used in Hb synthesis (erythrocyte basophilic punctations that appear on PBS, in a patient suspected of Pb poisoning, require the determination of plumbemia). In all the cases mentioned above, upon Prussian blue staining of the marrow aspirate, the ferritin aggregates generated in this way are called siderosomes, and their perinuclear arrangement in the erythrocyte precursors coincides with the physiological, perinuclear arrangement of mitochondria[11,12] (organelles in which 4 stages of heme synthesis take place, and in which Fe is stored excessively if it is not used properly). Etiological treatment of cirrhosis (antiviral, stopping alcohol) and its complications. In the case of lead poisoning, drug treatment with lead chelators is added and Zn administration is supplemented (Pb is substituted for Zn in δ-ALA Dehydratase and in Ferrochelatase, inhibiting enzyme activity; it can be eliminated from the enzyme sites by Zn supplementation). Pb chelators that can be administered orally are dimercaptosuccinic acid (succimer) and D-penicillamine.

10. Decreased hepatic production of EPO

The liver is the second organ involved in the production of EPO, after the kidneys. Decreases medullary production of erythrocytes. Treatment of cirrhosis.

11. Decreased renal production of EPO - renal damage in hepatorenal syndrome (HRS)

The progressive loss of renal function, during several weeks of HRS evolution, can also cause an EPO secretion deficiency. Decreases the bone marrow production of erythrocytes. It is added that uremia affects the functioning of platelets, increasing the risk of bleeding for this reason as well.[6] In HRS[15], renal flow decreases by redistributing the ECBV in the areas of collateral circulation that appear in portal hypertension, with the extravasation of a marked percentage of the ECBV in the ascites fluid. The splanchnic vasodilatation is favored by the increase in the production of NO at the level of the endothelial cells. Spontaneous bacterial colonization of the mesenteric nodes appears to be involved in this mechanism. Activation of the renin-angiotensin system fails to raise BP and improve renal perfusion. HRS is not triggered by diuretics, it has no pre-renal causes (hemorrhages, renal aa stenoses and so forth), renal causes (glomerulonephritis, vasculitis, ATN - given by aminoglycosides, iv contrast agents, NSAIDs, paracetamol, hemorrhages etc) or post-renal causes (obstructive stones etc). The curative treatment of HRS:[15]

LINE I: STOP BETA BLOCKER! Antibiotic treatment of spontaneous bacterial peritonitis!

For hospitalized patients: ***Option 1.*** midodrine (alpha1-sympathomimetic) orally 7.5-15 mg every 8 h + octeotride (somatostatin agonist with splanchnic vasoconstrictor effect) ivi -50 mcg/h or sc-100-200 mcg every 8 h **+** increase in oncotic pressure: administration of ivi of albumin 1g/kgc/day or max 100 g D1 and 2, then 25-50 g/kgc/daily until renal function normalizes or ***Option 2.*** terlipressin bolus ivi 1-2 mg every 4-6 h **+** administration of albumin ivi 1g/kgc/daily or max 100 g D1 and 2, then 25-50 g/kgc/daily until renal function normalizes.

In ICU (candidates for liver transplantation): ***Option 1*** - alpha-sympathomimetic: IV norepinephrine by syringe pump - 0.5-3 mg/h + administration of ivi of albumin 1g/kg/daily or max 100 g D1 and 2, then 25 each -50 g/kg/daily until the normalization of renal function; ***Option 2*** - splanchnic vasoconstrictor - vasopressin ivi on syringe pump - 0.01 U/min, with progressive dose

titration **+** ivi of albumin. The target is to increase the average BP by approx. 10-15 mmHg, above the threshold of 82 mmHg (see vasoconstrictor medication titration). Treatment can last up to 2 weeks.

LINE II: dialysis, TIPS, kidney transplant, liver transplant. Prevention of HRS: norfloxacin 400 mg po/daily chronic, or chronic prophylactic biseptol po.

12. Decreased renal production of EPO - renal damage through the precipitation of cryoglobulins

Renal damage is based on a type II cryoglobulin glomerulonephritis (especially in VLC with HVC, but also in VLC with HVB).[16-17] Type II cryoglobulins are immune complexes formed by monoclonal IgM (but also IgA and IgG), with rheumatoid factor (RF) activity, coupled with polyclonal Ig. The bone marrow production of erythrocytes decreases.[16,17]

13. Decreased renal production of EPO - renal damage due to amyloidosis

In systemic amyloidosis renal involvement is given by serum amyloid protein (AA amyloid). Serum amyloid protein is an acute phase reactant physiologically produced. Acute recurrent and sharpened chronic recurrent infections lead to its accumulation in tissues where it is deposited in the form of antiparallel beta chains, with the appearance of a pleated sheet; the HP microscopic examination of the kidney biopsy sample, stained with red Congo, it shows "green apple" birefringence in polarized light. The production of erythrocytes in the bone marrow is diminishing.[18]

14. Chronic inflammation

In chronic inflammation, iron is stored in tissues at the level of ferritin, not being available for heme synthesis. BC with hypochromic, microcytic anemia. Mechanism: IL-6 stimulates the release of hepcidin that degrades ferroportin: iron is no longer distributed from the enterocyte into the blood and is no longer released into the blood from the tissues where it is stored (liver, macrophages etc). Hepcidin also lowers transferrin levels. The result is: low serum iron, low transferrin, low TSC, decreased TIBC of transferrin, ferritin at the upper limit of normal or increased![19,20,21] This mechanism of simultaneous increase in iron storage and decreased transport of iron aims to lessen the available Fe, a trace element which could otherwise be used by various infectious agents - from this point of view is physiologically designed as protection mechanism.

15. Iron deficiency

Iron deficiency shows up through multiple mechanisms: 1. iron loss through repeated bleeding; 2. poor nutrition; 3. excessive use of PPIs leads to poor absorption of iron. BC with hypochromic, microcytic anemia. Perform sideremic profile (Fe, ferritin, TSC, TIBC). Inorganic iron combines with phytates, tannins, phosphates from food, and its absorption is lowerd in this way. It will be well absorbed if it is surrounded by a film of amino acids, vitamin C and carbohydrates. Organic iron needs an acid environment in the proximal duodenum for the solubilization of food and the release of ferric iron (Fe^{3+}) - rust - which will be enzymatically converted by ferrireductase into ferrous Fe (Fe^{2+}), the absorbable form of iron.[22,23] Microcytic, hypochromic anemia.

16. Poor transport of iron

Transferrin is synthesized in the liver. In cirrhosis there is a deficiency of its synthesis. Fe^{2+} taken up by ferroportin from the enterocyte, macrophage etc is brought into contact with 2 proteins hefaestin (ferrioxidase) and ceruloplasmin, thus being oxidized to Fe^{3+}, a form that is taken up by transferrin, a protein that transports iron in the blood stream.[21] A low concentration of the iron transporter - transferrin - causes a low delivery of iron to the hematogenous marrow: low Fe, TIBC, ferritin and increased TSC. Microcytic, hypochromic anemia. Treat cirrhosis and its complications.

17. Deficiency of vitamin B_{12}, folic acid (vitamin B_9)[24]

Is given by: low intake, malabsorption (edema of the GI mucosa, atrophy of the gastric mucosa due to excess antacid medication, intrinsic factor deficiency). Macrocytic, normochromic anemia - but if it is also associated with iron deficiency it can take the appearance of a normocytic, hypochromic anemia, and on PBS both macrocytes and microcytes with hypochromia are noticed. Treatment: im vit. B_{12}, according to known schemes, folic Ac. cpr 5 mg po. N.B. Beer drinkers can be deficient in vitamin B_{12}, but not in folic acid (beer contains folic acid). The im administration of vitamin B_{12}, in the case of the patient who presents a risk of bleeding (for instance anticoagulant

treatment) is preferable to be done by slow injection with a thin needle. Do not administer only folic acid to patients with vitamin B_{12} deficiency, because neurological deficiencies can be accentuated - both vit. B_{12}, as well as folic acid, through cytosolic biochemical processes, have a role in the methylation of nitrogenous bases, therefore, in the end, in cell replication, but only vit. B_{12} has a role in the mitochondrial burning in the Krebs cycle of the residues resulting from the degradation of fatty acids. In the absence of vit. B_{12} accumulating intermediate products of lipid metabolism are responsible for neurological degradation! BC is with hyporegenerative normochromic macrocytic anemia. PBS examination reveals macrocyte ovalocytes and hypersegmented neutrophils - with 5-6 lobes. BMA examination shows trilineage megaloblastic hyperplasia.

18. Copper deficiency

Multiple mechanisms are involved:
1. loss of Cu through repeated bleeding, exudative enteropathies, nephrotic syndrome;[25,26]
2. poor diet, excessive use of PPI;
3. excessive use of food supplements with Zn, creams for dental prostheses containing zinc - the intestinal absorption of Cu is low in the presence of excess Zn which induces the synthesis at the intestinal level of a protein of the Golgi apparatus, metallothionein, rich in cysteine, which preferentially binds Cu (as well as other trace elements and toxic metals) through thiol groups, increasing its intestinal excretion;[27]
4. Excessive use of Cu chelators in Wilson's disease;
5. Parenteral overload with Zn during chronic hemodialysis.[25]

Cu can be exploited in many ways by the human body:

a. for the absorption and use of iron, most likely by maintaining it in the oxidized state (ferric - Fe^{3+}); the complex hefaestin (or ferroxidase)-alpha-ceruloplasmin-Cu (wich also has a ferroxidase like activity) being in contact with ferroportin; Fe^{2+} released by ferroportin from cells into the blood stream is converted by this complex into Fe^{3+}, able to be bound by transferrin);[21,26]

b. in the transfer of electrons in fundamental metabolic and enzymatic pathways: cytochrome-c-oxidase (mitochondrial respiratory chain - oxidative phosphorylation);

c. Cu/Zn-dependent SOD (fighting oxidative stress);

d. Dopamine Beta-hydroxylase (catecholamine synthesis);

e. MAO (serotonin synthesis);

f. Tyrosinase (melanin synthesis);

g. Lysyloxidase (crosslinking of fibrils in collagen and elastin);

h. PGAA-monooxygenase (processing of peptide hormones and neuropeptides). 90% of Cu is carried in the serum by alpha-ceruloplasmin (which is also an acute phase protein).[25] Cu deficiency is usually associated with bicytopenia - leukopenia with macrocytic sideroblastic anemia (may mimic MDS), or with microcytic or normocytic anemia - or, rarely with pancytopenia. In the absence of Cu, the apoptosis of the late erythrocyte precursors in the bone marrow occurs with the increase of Fe turnover and its excessive accumulation at the level of the precursors in siderosomes (ferritin conglomerates located perinuclear). BC with low reticulocytes, PBS with dimorphic appearance on the red line (one normochromic and one hypochromic population of RBCs), macrocytosis and microcytosis. BMA: erythroid hyperplasia with ringed sideroblasts on Prussian blue staining, vacuolated erythroid and myeloid precursors, decreased myelo-erythroid ratio.[25,26] Neurological findings:[25] myeloneuropathy, optic neuritis, various sensory and motor neurological disorders. MRI examination reveals demyelinating lesions. Determination of serum copper is standard procedure. It is preferable to monitor cupremia, because alpha-ceruloplasmin is increased in inflammatory syndromes, being an acute phase protein, so false positive results may alter the interpretation![26] Treatment of the underlying disease is vital, ensure a rich Cu diet (red meat, mussels, shells, raisins, nuts, hazelnuts, cocoa, black chocolate etc), stop the administration of po Zn, give po elemental copper (8mg/day in W1, 6 mg/day in W2, 4 mg/day in W3, 2 mg/day weekly thereafter as needed) or injectable (elemental copper 2-2.4 mg slow ivi 2 h/day, 5-6 days consecutively, then weekly 2 mg slow ivi 2 h plus po Cu-gluconate - 2 tb. of 2 mg 2 times/day, in total 8 mg po/day, until the normalization of cupremia).[25,26] A differential diagnosis should be made with vitamin B_{12} deficiency.

19. Other deficiencies

Cirrhotic patients may have intake deficit or malabsorption for: vit. B_1 (thiamine - megaloblastic anemia +/- thrombocyopenia), vit. B_6 (pyridoxine - hypochromic microcytic anemia), vitamin B_2 (riboflavin - hypochromic microcytic anemia),

vitamin C (normocytic, macrocytic, microcytic - hypochromic anemia), vitamin E (tocopherol), proteins (in cirrhosis there is also increased catabolism of proteins; normocytic - normochromic anemia with anisocytosis and poikilocytosis).[13,26] Treatment: administration of the deficient substrate. Mechanisms: vitamin B_1 – is important in the conversion of pyruvate into Acetyl-coenzyme A, in the functioning of the Krebs cycle, facilitating the burning of fatty acids in the Krebs cycle, vitamin B_6 – is important in the synthesis of heme[28], vitamin C intervenes in the absorption of Fe, and together with folic acid is involved in the generation of tetrahydrofolate, vitamin B_2 is important in the absorption of Fe, vitamin E prevents hemolysis through oxidative stress, having an antioxidant role, the amino acids from the protein intake are important both in the absorption of iron and in the synthesis processes from the bone marrow precursors.[13,26]

20. AIHA

The cirrhotic patient presents an increased infectious risk, so he can develop infections with agents frequently involved in immune hemolysis:
1. Infections with EBV, Mycoplasma pneumoniae - usually cold-agglutinin-mediated AIHA;
2. Associated viral infections (in children with cirrhosis) or associated tertiary syphilis (in adults with cirrhosis) - Paroxysmal cold hemoglobinuria.[29,30]

Laboratory features:
1. DCT positive for C3b, C4b, C3dg, negative for IgG; IgM anti-i or anti-I antibodies present;
2. DCT positive for complement, negative for IgG; Positive Donath-Landsteiner Ab - Anti-P IgG Ab, positive Donath-Landsteiner biphasic test 3. BC with reticulocytosis, biochemistry with increased IB and LDH, low haptoglobin, agglutination on blood film of red blood cells at room temperature in cold-agglutinin-mediated AIHA. This agglutination won't occur if the blood is kept warm. Ensure the etiological treatment of anemia and also AIHA treatment. AIHA with cold antibodies (except for the situation where the thermal amplitude of the antibodies is increased) and paroxysmal cold hemoglobinuria do not respond to corticosteroid therapy![29,30]

21. Microangiopathic hemolytic anemia

1. On the background of severe liver failure from end-stage cirrhosis, cholesterol is no longer taken up by the liver to be used metabolically, the excess cholesterol from the red blood cell membranes inhibits LAT, the key enzyme involved in the repair of erythrocyte membranes, and causes their deformation, lysis (the appearance of macrocytes, acanthocytes) that cannot pass the sinusoid capillaries (splenic etc) and are mechanically hemolyzed. LAT transfers acyl groups from membrane reserves of acyl-carnitine to acyl-CoA; from acyl-CoA these acyl groups are attached to lysophospholipids with membrane lytic potential in order to disable them - lysophospholipids are fatty acid residues generated by the physiological mechanism of removing peroxidized fatty acids from the erythrocyte membrane.[31] Also, in liver cirrhosis, echinocytes appear by attaching abnormal HDL molecules to RBC membrane proteins.[31]
2. Excess acetaldehyde in erythrocyte membranes, produced as a result of alcohol metabolism, in ethylic cirrhosis, causes the appearance of macrocytosis independent of B_{12} and /or folate deficiency,[31] macrocytes showing increased membrane fragility; add to this the fact that in the serum of patients with liver damage of alcoholic etiology, antibodies (IgA and IgM) were detected against some erythrocyte proteins conjugated with acetaldehyde.[12]
3. Even if the diet is normal, alcohol may block the mobilization and use of folic acid, without influencing its absorption, macrocytosis being also generated by this mechanism.[32]
4. Prothrombotic status in cirrhosis also exists through the association of some of the conditions below: hypoperfusion of the congested portal bed, reduced synthesis of anticoagulants (of AT-III, protein C, protein S) in cirrhosis,[33,34] reduced clearance of activated coagulation factors (IXa, Xa, XIa), septic status due to immunosuppression (endothelial lesions produced by endotoxins of intestinal origin), shunt of ascites fluid (rich in plasminogen activators, plasmin, collagen) in the venous circulation (LeVeen shunts), the appearance of a liver neoplasm in a cirrhotic patient, shock. Combinations of these factors determine the appearance of DIC in the cirrhotic patient.[35,36] Coagulopathy can lead to portal vein thrombosis and/or suprahepatic vein thrombosis, DVT, rarely arterial thrombosis (especially in protein S deficiency). The synthesis of proteins C, S is dependent on vitamin K. In the first stage of vitamin K deficiency, the thrombotic risk predominates. Thrombotic risk, depending on the associated pathology of the cirrhotic patient, can be increased by additional thrombogenic risk factors: advanced age, pregnancy, dyslipidemia, obesity, diabetes, increased blood pressure, smoking, coffee,

sedentary lifestyle, immobilization, insufficient water intake, surgical stress, infections (HIV etc), hyper- or hypo-thyroidism, nephrotic syndrome, chronic kidney disease, inflammatory bowel diseases, APLS, hyperhomocysteinemia, thrombophilia, polyglobulia, leukostasis syndromes, acute promyelocytic leukemia, hyperviscosity syndromes (MM, WM), NPH, the use of certain drugs - contraceptives, heparin, L-Asparaginase, thalidomide, lenalidomide, bevacizumab and so forth. PBS detects schizocytes (fragmented red blood cells), echinocytes (spiculated RBC with blunt, uniform projections), acanthocytes (spiculated RBC with with multiple irregular spikes, unevenly ditributed). The appearance of echinocytes, acanthocytes on the PBS, as well as hypoglycemia in liver cirrhosis indicates the impossibility of the liver to function normally (it can no longer take cholesterol to integrate it into bile salts, lipoproteins etc, it no longer has glycogen reserves, it can no longer perform glycogenolysis and can no longer do gluconeogenesis) representing an urgent indication for liver transplantation.[4,31]

Treat cirrhosis and its complications.

Ensure supportive treatment: PRBC transfusion.

In case of DIC, treat DIC, the infection etc.

Targeted treatment, addressed to additional risk factors is required.

N.B. A special case of microangiopathic hemolytic anemia in liver cirrhosis of viral etiology is Thrombotic Thrombocytopenic Purpura (TTP), a medical emergency in which, through post-infectious autoimmunity phenomenon, the patient develops blocking Abs against the enzyme involved in the cleavage of the VW factor macromolecule (ADAMTS13). The most common anti-ADAMTS13 Abs are IgG4 type, less often are IgA or IgM types. ADAMTS13 does not have a low level in liver failure, being synthesized in the hepatic stellate cells and not in the hepatocyte, but also in the kidney (by podocytes) or at the endothelial level.[37] Being an acute phase protein, it can increase in liver failure, on the background of inflammation, stellate cells reacting to liver injury by activation and proliferation. The activation of the macromolecule of the VW factor through shear stress in the precapillary arterioles, not being followed by enzymatic cleavage into smaller size monomers of the VW factor, allows platelets to bind to the macromolecule and favours the appearance of unstable thrombi, thus triggering trasient tissue ischemia and mechanical hemolysis.

TTP involves:

1. microangiopathic hemolytic anemia (CB with anemia, PBS with schizocytes, increased LDH and IB);
2. thrombocytopenia (through consumption on the background of thrombogenesis);
3. fever;
4. kidney damage;
5. brain damage (epileptic seizures, behavioral disorders, TIA manifestations, stroke etc) - the classic diagnostic pentad, which is not always complete (it can also be a dyad - anemia and thrombocytopenia, a triad or a tetrad).

Required tests in order to certify the diagnosis are: ADAMTS13 antigen dosage, ADAMTS13 activity level (level below 10% is diagnostic for TTP), as well as anti-ADAMTS13 antibodies.

TTP imposes emergency hematological treatment: corticotherapy, plasmapheresis with plasma exchange in parallel with the administration of Caplacizumab, ivi on the first day, then sc, 1 dose daily. In the absence of Caplacizumab, Rituximab can be used at a rate of 1 administration/week. Cablivi or Rituximab administration on the one hand reduces the need for plasmapheresis/plasma exchange, on the other hand it can be kept as a complementary therapy in cases refractory to corticotherapy and plasmapheresis/plasma exchange. The treatment is done until the platelets return to normal in two consecutive determinations.

22. Zieve syndrome

Zieve syndrome was first described in 1958 by Leslie Zieve in patients who presented the following triad: alcoholic liver disease (hepatitis, alcoholic cirrhosis), hemolytic anemia, hypertriglyceridemia. It appears in alcoholic patients after an acute episode of excessive alcohol consumption.[12] The pathophysiology of the condition is not yet fully understood. Several mechanisms are currently described: **1.** Hypertriglyceridemia has an important role in hemolysis. The acute excess of alcohol releases a large amount of TG in the blood stream that cannot be efficiently processed by gluconeogenesis, especially since the pancreatic alpha cells, which physiologically produce glucagon, are also affected by alcohol.[38] Zieve indicated that hemolysis occurs on the background of hypertriglyceridemia through the accumulation of some abnormal lipids, such as lysolecithin, involved in the degradation of the erythrocyte membrane.[39] Lysolecithin, lysocephalin are blamed for triggering and exacerbating hemolysis.[39] The problem was that transfused RBC were also hemolyzed in patients with Zieve syndrome

(Balcerzak et al., 1968), leading to the assumption that there should be also additional inner causes of hemolysis. Subsequent research indicated that the decrease in the level of tocopherol (vitamin E), a fat-soluble vitamin with a major antioxidant role, deficient due to malabsorption and/or malnutrition in chronic alcoholism, has an important role in hemolysis. The deficiency of vitamin E involves an increase in oxidative stress, with a decrease in the level of polyunsaturated fatty acids, affecting the fluidity of the erythrocyte membrane (increasing the fraction between the concentration of saturated fatty acids and cholesterol reported to the concentration of polyunsaturated fatty acids), the result being a decrease in its plasticity and finally, hemolysis. What about the inner causes of hemolysis? At low concentrations of vitamin E, on the one hand the oxidation of reduced erythrocyte glutathione is accelerated, but on the other hand the stability of pyruvate kinase (the key enzyme of energy metabolism, of erythrocyte anaerobic glycolysis) is is endangered, these latter two constituting yet another mechanism of non-immune hemolysis.[38,39] In this case it shapes the picture of mixture of simultaneously aquired G6PD and PK deficiency. Hematologically, anemia is detected on the BC, spherocytes and acanthocytes are observed on the PBS (Zieve syndrome is one of the few causes of spherocytosis[40] along with: AIHA, hereditary spherocytosis, Wilson's disease, Clostridium perfringens - also known in the past as C. Welchii - sepsis). DCT, ICT are negative. Biochemical findings: a marked increase of both DB due to the liver damage of alcoholic etiology, but also of hemolysis markers (IB, LDH), significant increase of triglycerides. Treatment: stopping alcohol consumption is a must, PRBC transfusion, administration of glutathione and vitamins (including vitamin E),[38,39] treatment of alcoholic cirrhosis and complications, and in case of severe hypertriglyceridemia, in patients who already have a history of pancreatitis and intracerebral hemorrhages, emergency plasmapheresis will be performed.

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