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TOPICS

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* *The publication is intended to all medical students of UMF Carol Davila, PhD students – UMF Carol Davila, young residents and researchers.*

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Review

Deficitul de fier şi anemia în insuficienţa cardiacă

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Cuvinte cheie: *anemie, deficit de fier, feriprivie, insuficienţă cardiacă, corecţie deficit fier, ferric carboxymaltoză, ferric derisomaltoză.*

Abrevieri: **ACE**- enzima de conversie a angiotensinei, **FE** – fracţie de ejecţie, **IC**- insuficienţă cardiacă, **i.v.** – intravenos, **RFG** – rata de filtrare glomerulară, **MO** – măduvă osoasă.

Abstract

Sindromul complex al insuficienţei cardiace are faţete multiple, etiologice, patogenice, clinice şi evolutive. În evoluţia pe termen lung (clinică) se asociază cu multiple tipuri de comorbidităţi, care au impact pe morbiditate, calitatea vieţii şi mortalitate.

Deficitul de fier şi anemia în insuficienţa cardiacă (IC) a devenit un subiect major de cercetare complexă în ultimii 10-15 ani. Studiile epidemiologice au semnalat (demonstrat) că deficitul de fier se întâlneşte la aprox. 50% din pacienţii cu IC şi FE redusă şi că este independent asociat cu scăderea capacităţii funcţionale, calitatea vieţii şi creşterea mortalităţii[1]. La pacienţii cu IC acută (sau decompensată acut) deficitul de fier s-ar întâlni până la 80%. Studiile au mai semnalat că deficitul de fier se poate însoţi sau nu de anemie, iar administrarea unor preparate de fier i.v. corectează anemia, restabileşte valorile sideremiei şi ameliorează simptomele şi capacitatea de efort la pacienţii cu IC şi FE redusă[2].

Homeostazia fierului

Dereglarea homeostaziei fierului este strâns legată de deficitul de fier şi insuficienţa cardiacă.

Fierul este un microelement esenţial pentru structura şi funcţia celulelor, ţesuturilor şi este implicat în producerea de energie.

În organismul uman se găsesc 3-4 g de fier, majoritatea în mioglobina, măduva osoasă hematogenă, hemoglobină şi în constituţia enzimelor celulare. Fierul provine – în totalitate – prin absorbţie în tubul digestiv (prin enterocit la nivel duodenal şi jejunul proximal) şi se integrează în procesul de hematopoeză. Homeostazia fierului este asigurată prin 3 procese interconectate: absorbţie, fier utilizat (în hematopeză, la nivel celular pentru producerea de energie) şi fier stocat (în ficat, splină).[3] În *homeostazia fierului* intervine nesemnificativ, pierderea de fier (în condiţii fiziologice).

Patru elemente (markeri) sunt implicate şi intercorelate în homeostazia fierului, nivelul fierului seric şi în măduva osoasă: feritina, transferina şi hepcidina[4].

Valoarea normală a fierului seric este apreciată de < 13 μmol/L şi este o variabilă multiplu dependentă.

Fierul absorbit prin enterocit este stocat în celule sub formă de feritină şi poate ajunge în circulaţie cu ajutorul unei proteine transportoare.

Feritina este o proteină produsă la nivelul ficatului, măduvei osoase și splină. Structural este un complex între hidroxiferos și apoferitină. Are rol de stocare a fierului la nivel celular și eliberarea când este necesar. Feritina este astfel marker al depozitelor de fier și concentrația sa în plasmă, se corelează cu depozitul de fier[4]. Când nivelul plasmatic al fierului este scăzut, se eliberează fier din depozitele de feritină – prin intermediul feroportinei.

Feritina acționează și ca un reactant de fază acută și nivelul feritinei crește în procesele inflamatorii.

Transferina este o glicoproteină produsă în cea mai mare parte în ficat. Asigură legarea și transportul fierului și este singura sursă fiziologică de fier disponibil pentru metabolism. Gradul de saturare al transferinei (TSAT) reflectă biodisponibilitatea fierului.

Hepcidina este un hormon peptidic produs de ficat și macrofage și are un rol important în homeostazia fierului reglând absorbția fierului din tubul digestiv. Nivelul hepcidinei serice se corelează cu nivelul fierului plasmatic și feritinei. Fierul plasmatic crescut va crește secreția de hepcidină care, cu ajutorul feropontinei, duce la scăderea eliberării de fier din enterocite și țesutul reticuloendotelial. Când nivelul plasmatic al hepcidinei este foarte scăzut, se produce supraîncărcare cu fier (hemosideroză, hemocromatoză) datorită creșterii feroportinei care induce aflux de fier din celulele care îl stochează și crește absorbția de fier.

Feroportina activează ca un "exportator" major al fierului, transportând fierul absorbit din enterocite în circulație.

Definirea termenilor: anemia și deficitul de fier

Dereglarea homeostaziei fierului în una din componentele sale poate conduce la deficit de fier (frecvent) sau la exces de fier (hemocromatoză, hemosideroză). Deficitul de fier și efectele sale pe hematopoieză conduc relativ precoce la anemie. Deficitul de fier este un element major în evoluția insuficienței cardiace, iar anemia poate însoți sau nu deficitul de fier[5].

Relația deficit de fier – anemie în insuficiența cardiacă, necesită definirea (acceptată) a termenilor.

Anemia este definită conform recomandărilor OMS și a societăților academice de specialitate – prin valorile hemoglobinei < 12 g Hb/dl – pentru femei și < 13 g Hb/dl pentru barbați. În raport cu aceste valori, anemia poate avea grade diferite de severitate.

Deficitul de fier este definit printr-o formulă care include markeri funcționali: valori feritină < 100 µg/L sau valori feritină între 100-299 µg/dl și saturația transferinei < 20% (7). Formula ar avea sensibilitate de 80% și specificitate de 72% pentru detectarea deficitului de fier[1].

Formula actuală a fost aprobată prin consens și este inclusă în ghidurile de insuficiența cardiacă ACC/AHA, ESC - respectiv în 2020 și 2021[5,6].

Formula deficitului de fier recomandată și folosită în studii de insuficiență cardiacă ar avea unele limitări. Feritina, ca marker al depozitului de fier celular (funcțional) poate avea valori crescute în condiții de injurie celulară dar și în condiții inflamatorii; valori înalt crescute ale feritinei, folosit ca unic marker, ar masca deficitul de fier. A fost propusă o formulă alternativă: fier seric < 13 µmol/L și saturația transferică < 20% ar avea sensibilitate de 94% și specificitate de 88% pentru deficitul de fier. Într-un studiu de cohortă – 4426 pacienți cu insuficiență cardiacă – aplicarea formulei TSAT < 20% și Fe seric < 13 µmol/L s-a asociat, sub aspect prognostic, de creșterea riscului de mortalitate la 5 ani (21%)[7].

Relațiile între deficitul de fier și prezența anemiei în insuficiența cardiacă nu sunt directe. Anemia nu poate fi utilizată ca screening pentru diagnosticul deficitului de fier; aprox. 1/3 din pacienții cu deficit de fier nu au anemie [8].

Diagnosticul și cauzele (condițiile) care duc la deficit de fier și anemie în insuficiența cardiacă

Evaluarea deficitului de fier și anemiei în insuficiența cardiacă se efectuează inițial prin screening la grupele de lucru. Ghidul de diagnostic și tratament al IC acute și cronice, recomandă screening periodic pentru toți pacienții cu IC (recomandare I-C)[6].

Diagnosticul deficitului de fier în IC se bazează pe teste simple: feritina serică și saturația transferinei (TSAT). Cercetarea fierului medular este necesară numai în cazuri speciale.

Diagnosticul anemiei se bazează pe un test rapid: dozarea hemoglobinei (valori scăzute). Prezența anemiei nu este un marker corect pentru identificarea deficitului de fier dar valori scăzute ale hemoglobinei pot fi determinate de deficitul de fier[1]. Prezența anemiei la pacienții cu IC, necesită o evaluare, cu parametri multipli:

- Hemogramă completă (inclusiv pe lamă pentru indici eritrocitari, inclusiv reticulocite);
- Aprecierea feritinei serice și a saturației transferinei (STAS);
- Dozarea vitaminei B_{12} și a folaților serici;

- Markeri de inflamaţie (Prot C reactivă, feritina);
- Aprecierea funcţiei renale (BUN, creatinină, RFG).

Explorările biologice semnalate, împreună cu datele clinice, permit eliminarea altor tipuri de sindroame anemice posibile şi în IC şi care nu sunt legate de deficitul de fier.

Cauzele potenţiale al deficitului de fier şi anemie în IC sunt multiple dar în principal sunt aportul insuficient de fier (deficit nutriţional), depozitarea de fier (MO, ficat, splină etc) şi pierderea de fier[9].

- *Aportul nutriţional* de proteine, fier este un prim factor atât de deficit de fier cât şi anemie. Deficitul nutriţional se accentuează cu progresia IC, până la pierdere ponderală severă şi caşexie cardiacă. Aportul de proteine poate fi limitat de scăderea apetitului, staza digestivă. Hipoproteinemia, special nivelul albuminemiei este un predictor independent de deficit de fier[9].
- *Congestia venoasă* prezentă în IC dreapta se traduce la nivel digestiv, prin creşterea grosimii peretelui intestinal, scăderea absorbţiei de elemente nutritive – inclusiv fier – şi malabsorbţie.
- *Starea inflamatorie cronică* şi creşterea citokinelor inflamatorii, interleukina 6 (IL-6, TNF-α) conduce la creşterea depozitelor de fier şi realizează anemie de tip inflamator. Nivelul hepcidinei creşte în stările inflamatorii şi intervine în absorbţia fierului[10]. Suma citokinelor inflamatorii influenţează direct statusul fierului, independent de hepcidină şi conduce la creşterea depozitelor de fier şi anemie de tip inflamator[11].
- Pacienţii cu IC clasele II-IV NYHA, primesc de *regulă medicaţie optimă de tratament*: inhibitori de angiotensină, betablocante, inhibitori de mineralo-corticoizi şi inhibitori SGLT2. Inhibitorii ACE ar bloca parţial eritropoeza, iar betablocantele ar induce inhibiţia precursorilor eritroizi, condiţii care conduc la perturbarea homeostaziei fierului. În plus, o parte din pacienţii cu IC, necesită tratament anticoagulant (AVK, DOAC) sau/ şi antiplachetare pentru prevenţia tromboembolismului sistemic (fibrilaţia atrială) sau a evenimentelor trombotice pe leziunile aterosclerotice "vulnerabile". Dintre antiplachetare – în special aspirina poate creşte riscul micro sângerărilor pe tubul digestiv, iar anticoagulantele orale şi sângerări semnificative clinice digestive sau cerebrale[9].
- *Disfuncţia renală de grade diferite* este o condiţie relativ frecventă la pacienţii cu IC clasele III-IV NYHA. Vârsta înaintată sau/ şi prezenţa diabetului zaharat pot agrava disfuncţia renală. Bolnavii cu hemodializă cronică au sindrom inflamator care blochează fierul în MO, splină etc.
- *Pierderea de fier* prin sângerări nu trebuie subestimată la evaluarea deficitului de fier sau/ şi anemiei, la pacienţii cu insuficienţă cardiacă cronică şi tratament farmacologic de lungă durată.

Consecinţele deficitului de fier şi anemiei la pacienţii cu IC

Fierul este o componentă cheie a proteinelor structurale (celulare, tisulare) şi un cofactor pentru activitatea enzimatică la nivel celular. *Fierul este esenţial pentru funcţia mitocondrială şi respiraţia aerobă*. Cardiomiocitele conţin mai multe mitocondrii pentru a produce energia adecvată şi devin astfel particular sensibile la depleţia de fier şi aportul de oxigen[1]. În condiţii experimentale s-a observant că deficitul de fier afectează contractilitatea miocardică prin scăderea funcţiei mitocondriale.

Deficitul de fier se asociază cu modificări subclinice la nivelul structurilor cardiace, remodelare adversă şi disfuncţie ventriculară. Modificări structurale similare se produc şi la nivelul muşchilor scheletici prin reducere de substrat şi generare de energie[12].

Deficitul de fier creşte rezistenţa la eritropoetina şi reduce hematopoeza. Celulele în diviziune rapidă (ex. celulele hematopoetice) şi cele cu necesar înalt de energie (mitocondrii, miofibrile) devin vulnerabile prin reducerea aportului de oxigen şi producere de energie (Figura 1).

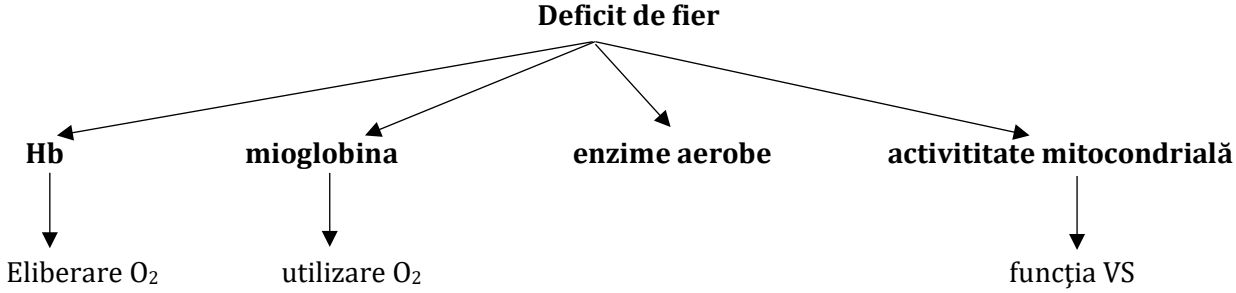

Figura 1. Efecte detrimentale ale deficitului de fier pe multiple componente de utilizare O_2

În sinteză, deficitul de fier şi anemia au efecte negative (deletere) în folosirea oxigenului la nivel celular şi tisular[12]:
- Prezenţa anemiei (scăderea hemoglobinei) reduce transportul de O_2 şi eliberarea la celule
- Deficitul de fier reduce enzimele aerobe şi scade utilizarea oxigenului
- Deficitul de fier reduce activitatea mitocondrială, producerea de energie şi subsecvent disfuncţie cardiomiocitară.

Din punct de vedere clinic, deficitul de fier, asociat sau nu cu anemie, conduce la reducerea de energie la nivel cardiomiocitar, scăderea contractilităţii cardiace, agravarea semnelor şi simptomelor de insuficienţă cardiacă[13].

Reducerea (corectarea) deficitului de fier şi anemiei conduc la pacienţii cu ICrEF la ameliorarea simptomelor, creşterea capacităţii funcţionale, ameliorarea calităţii vieţii şi reducerea spitalizărilor şi mortalitatea cardiovasculară? Sunt întrebări la care se poate răspunde după analiza studiilor care au folosit administrare de fier în insuficienţa cardiacă.

Tratamentul deficitului de fier – anemie la pacienţii cu insuficienţă cardiacă

Incidenţa mare a deficitului de fier la pacienţii cu IC (aprox. 50%) a condus la ideea tratamentului cu fier în IC. Obiectivul primar al tratamentului este repleţia de fier şi corectarea pe termen lung a deficitului de fier având în vedere persistenţa cauzelor necontrolabile ale deficitului în IC cronică. Corecţia deficitului de fier poate corecta şi anemia, când este prezentă, ambele condiţii intervenind variabil la agravarea insuficienţei cardiace.

În ICrEF tratamentul cu fier, se adaugă tratamentului farmacologic actual optim şi bazat pe administrarea a "4 piloni": IEC-A, betablocante, inhibitori de mineralocorticoizi şi inhibitori SGLT2. Când este prezentă congestia, tratamentul cu diuretice devine obligatoriu[6].

Numeroase cercetări, studii clinice randomizate, meta-analize, au urmărit efectele administrării de fier la pacienţii cu ICrEF, pentru perioade variate de timp (luni, ani). Global, obiectivele urmărite se referă la eficienţă şi siguranţă şi în special, efectele administrării de fier asupra unor elemente, ca de exemplu:
- Scorul global funcţional
- Clasa NYHA a IC
- Valorile NT pro-BNP
- Capacitatea de efort şi timpul de mers 6 min
- Spitalizare şi respitalizarea pentru IC
- Calitatea vieţii
- Mortalitatea cardiovasculară.

În studiile publicate au fost urmăriţi mai puţin parametrii hematologici după tratamentul de substituţie a deficitului de fier.

Preparatele de fier utilizate în studii au fost (şi sunt) preparatele orale sau preparatele de fier administrate i.v.

Fierul per os este prescris, de regulă, în anemia feriprivă, pentru o perioadă determinată (1-3 luni), în raport de corectarea anemiei şi a deficitului de fier. Preparatele de fier mai frecvent utilizate sunt gluconatul feros, sulfatul feros, fumaratul feros.

Administrarea de fier per os la pacienţii cu ICrEF şi deficit de fier, poate corecta lent (1-3 luni), deficitul de fier şi anemia. Compensarea cardiacă se poate realiza de asemenea lent.

La pacienţii cu IC, absorbţia fierului este limitată, datorită creşterii grosimii peretelui intestinal (prin congestie) şi modificării nivelului hepcidinei [11]. Creşterea citokinelor proinflamatorii contribuie la creşterea inadecvată a nivelului hepcidinei, care bolchează absorbţia de fier[11]. În plus, suplimentarea cu fier poate creşte independent hepcidina care este hiper expresată în IC.

În studiul IRONOUT, administrarea de fier per os în ICrEF şi deficit de fier, a crescut modest saturaţia transferinei şi a feritinei, dar fără creşterea capacităţii de efort a pacienţilor[14].

În Ghidul 2021 ESC de insuficienţă cardiacă, precum şi în Ghidul 2022 ACC (AHA), administrarea de fier per os, nu este recomandată în deficitul de fier din ICrEF (recomandare clasa III), în absenţa unor dovezi semnificative de eficienţă [6,15].

Introducerea preparatelor de fier cu administrare i.v. reprezintă un progres major în tratamentul ICrEF cu deficit de fier. Iniţial a fost folosit Fier dextran dar efectele sale adverse – în special reacţiile anafilactice – au condus la renunţarea administrării sale în practică.

În prezent sunt folosite 3 produse de Fier pentru administrare i.v. fier: sucroza (doză 200 mg), ferric carboxymaltoză (FCM – doză 500-1.000 mg) şi ferric derisomaltoză (FDM – doză < 2.000 mg)[1]. *Cele mai multe studii clinice de eficienţă şi siguranţă, s-au efectuat cu FCM la pacienţii cu ICrEF şi deficit de fier.*

Preparatele de fier pentru folosirea i.v., se deosebesc prin dozele administrate (iniţial sau pentru tratament prelungit), durata administrării i.v. (minute – ore) şi rezultatele pe corecţia fierului şi a insuficienţei cardiace.

Folosirea preparatelor de fier cu administrare i.v. evită calea digestivă (absorbţia fierului scăzută în

IC), realizează un efect mai rapid decât la administrarea per orală şi în plus au o toleranţă foarte bună şi reacţii adverse minime.

Dovezile privind eficienţa şi siguranţa administrării de FCM la pacienţii cu ICrEF şi deficit de fier au fost aduse de numeroase studii clinice randomizate şi de repetate meta-analize, care la analiza finală au adus rezultate în parte diferite.

Două studii iniţiale FAIR-HF şi CONFIRM[2,16] *au adus rezultate aproape similare. Studiul FAIR-HF* randomizat (459 pacienţi) a folosit o doză iniţială de 200 mg FCM, eventual repetată la 4 săptămâni la pacienţii cu ICrEF şi deficit de fier. La 26 săptămâni, la aprox. 50% din pacienţi s-a remarcat o ameliorare semnificativă. Ameliorarea scorului global de evaluare a pacientului a fost înregistrat la 28% versus placebo (p < 0,001). A fost consemnată îmbunătăţirea clasei NYHA la 47% din pacienţi şi ameliorarea testului de mers 6 minute. Efectele administrării FCM au fost omogene atât la pacienţii anemici cât şi în absenţa anemiei[2].

În studiul CONFIRM-HF – studiu multicentric, randomizat cu 304 pacienţi cu IC clasele III-IV, FE < 45% şi creşterea NT pro-BNP, s-a folosit iniţial doză mai mare 500-1.000 mg FCM, urmată de doză de întreţinere de 500 mg la 12, 24, 36 săptămâni. Rezultatele au fost similare cu cele semnalate în studiul FAIR-HF, dar în plus a fost consemnată reducerea spitalizării pentru IC[16].

O meta-analiză din 2018 publicată de Anker S.D şi colab – promotori ai folosirii FCM în tratamentul ICrEF şi deficitului de fier - a adus rezultate noi. Analiza rezultatelor la 839 pacienţi, din care 504 randomizaţi cu FCM versus placebo a arătat ameliorarea simptomelor, capacităţii funcţionale, calităţii vieţii, dar şi reducerea spitalizărilor recurente şi a mortalităţii cardiovasculare[17].

Studii mai recente, AFFIRM-HF, IRONMAN şi HEART-FID (2022 şi 2023) au cercetat eficienţa şi siguranţa administrării de FCM în ICrEF, urmărindu-se obiective mai puternice (strong). Rezultatele au fost nuanţate, în parte diferite faţă de studiile mai vechi.

Studiul AFFIRM-HF a urmărit eficienţa FCM (doza iniţială 1.000 mg) la 1.132 pacienţi spitalizaţi pentru IC acutizată. Obiectivul primar a fost spitalizarea pentru IC şi mortalitatea cardiovasculară la 1 an.

Rezultatele pe obiectivul primar nu au fost diferite între grupul cu tratament FCM şi placebo: prima spitalizare sau deces cardiovascular 32% la pacienţii cu FCM şi 39% pentru placebo (HR 0,29, p < 0,03). A fost semnalată de asemenea şi creşterea hemoglobinei cu 0,8 g/dl faţă de 0,3 g/dl în grupul placebo[18].

Studiul IRONMAN – studiu prospectiv randomizat la 1.137 pacienţi cu ICrEF şi deficit de fier, s-a efectuat în condiţii de ambulator. Doza iniţială de fier a fost deriso maltoza aprox. 2.000 mg; perioada de urmărire 2,7 ani, obiectivul primar a fost similar celui din studiul AFFIRM.

Concluziile studiului sunt parţial diferite de cele semnalate în AFFIRM: pe reducerea spitalizării pentru IC şi a mortalităţii cardiovasculare[19].

Studiul HEART-FID – randomizat, dublu orb, a fost efectuat la pacienţi cu ICrEF şi deficit de fier, urmărindu-se 12 luni rezultatele între grupul cu FCM (1532 pacienţi) şi *placebo* (1533 pacienţi). După 12 luni spitalizarea pentru IC a fost consemnată la 297 vs 332 pacienţi şi mortalitatea la 8,6% *vs.* 13,2% respectiv la grupele studiate. Autorii au precizat în concluzii – nu s-a găsit nici o diferenţă între grupul de pacienţi trataţi cu FCM *versus placebo* în ceea ce priveşte obiectivul compus: deces şi spitalizare pentru IC[20].

O meta-analiză foarte recentă (2023) însumând 12 studii şi 4.376 pacienţi cu ICrEF, dintre care 45,3% au primit fier i.v. *versus* tratament standard, consemnează următoarele concluzii:

- Reinternarea pentru IC semnificativ mai mică la pacienţii trataţi cu fier i.v. (FCM) *versus* grupul martor – RR 0,73 p = 0,026
- Nicio diferenţă semnificativă pe mortalitatea globală şi cardiovasculară – RR 0,88 p = 0,15
- Rata infecţiilor similară la ambele grupe (p < 0,7)[21].

Concluzii

Rezultatele aparent diferite în studiile analizate pot avea explicaţii diferite privind loturile studiate, protocoalele studiilor, particularităţile specifice de grup, dar ajung la o concluzie comună: tratamentul cu fier i.v. (FCM) la pacienţi cu ICrEF şi deficit de fier, aduce ameliorarea simptomelor, a capacităţii funcţionale, posibil ameliorarea NT pro BNP şi revers remodelarea, reducerea primei spitalizări pentru IC sau respitalizare. Pentru reducerea mortalităţii cardiovasculare la pacienţii trataţi cu fier i.v. nu sunt dovezi suficiente pentru confirmare.

Ghidul 2021 ESC pentru diagnosticul şi tratamentul insuficienţei cardiace acute şi cronice, recomandă suplimentarea cu ferric carboxy maltoza (FCM) la pacienţii cu IC simptomatică recent spitalizaţi – FE < 50% şi deficit de fier – pentru a reduce riscul de spitalizare (clasa II a)[6].

În acelaşi Ghid 2021 ESC, se recomandă screening periodic pentru evidenţierea anemiei şi a deficienţei de fier, la toţi pacienţii cu IC (clasa I).

Agenţii stimulatori ai eritropoezei, folosiţi în tratamentul anemiei la pacienţii cu insuficienţă renală cronică dializaţi, au fost testaţi şi la pacienţii cu ICrEF, deficit de fier şi anemie moderată (studiul RED-FER). Administrarea de darbepoetină (2278 pacienţi randomizaţi) au avut efecte neutre pe supravieţuire şi pe evoluţia insuficienţei cardiace. Efectele adverse ale darbepoetinei au fost importante: creşterea ratei evenimentelor tromboembolice (13,5% *vs*. 10% p < 0,009) şi creşterea riscului de Stroke ischemic[21].

În prezent tratamentul anemiei cu stimulatori ai eritropoezei la pacienţii cu ICrEF şi deficit de fier a fost abandonat. În Ghidul 2021 ESC este prevăzută recomandarea clasa III/c[6].

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Short communication

Potential Causes of Anemia in Hepatic Cirrhosis

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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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Literature Review

Prenatal Diagnosis of Thalassemia

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Abstract

Thalassemia is a group of inherited blood disorders that affect the production of hemoglobin. The main components of hemoglobin consist of heme and globin (α-globin and β-globin). Thalassemia occurs when there is a mutation in the genes responsible for producing these globin chains, leading to an abnormal or insufficient production of one or both types of globin. Depending on the affected globin genes, α- and β-thalassemias can be identified. Depending on the genotype, the clinical presentation of the patient can vary from asymptomatic to severe and also lethal courses. The main and also leading symptom is microcytic hypochromic anemia. Without a treatment, increased erythronpoiesis can induce extramedullary hematopoiesis, hepato-splenomegaly and growth disturbances.

In the context of α-thalassemias distinctions are made among the minor and minima form (expressed by few symptoms), HbH disease (manifesting mild to moderate symptoms) and Hb Barts hydrops fetalis syndrome (showing severe symptoms and also often resulting in perinatal death). The choice of treatment depends on the specific clinical presentation and closely mirrors the approach taken for β-thalassemias.

In the case of β-thalassemias, a differentiation is made between the minor form (also with few symptoms) and the homozygous major form. Without a treatment the latter leads to severe outcomes in childhood. The symptomatic therapy involves transfusions and efforts are made to mitigate the life-limiting complications of secondary iron overload by administering iron chelators (e.g. Deferoxamine). Causal therapy is possible through a stem cell transplant, or experimentally through gene therapy.

Introduction

Various mutations affecting hemoglobin have been discovered so far. Thalassemias can lead to severe anemia from an early age, and without regular blood transfusions, they can result in death within the first year of life. Prenatal detection of thalassemia is a crucial component of preventive medicine, typically relying on invasive diagnostic tests during the initial two months of pregnancy[1]. However, these diagnostic techniques carry a small yet noteworthy risk of fetal loss, around 1%. Molecular diagnostic approaches for genotyping thalassemias have been developed, employing PCR methods and advanced high-throughput technologies. Another alternative method involves noninvasive testing, utilizing cell-free fetal DNA (cffDNA) obtained from a maternal blood sample, effectively eliminating the risk of miscarriage[2].

Epidemiology

Thalassemia has a global incidence of approximately 4.4 cases per 10,000 live births. It is noteworthy that both males and females inherit the responsible gene mutations equally, as thalassemia follows an autosomal pattern of inheritance, showing no gender preference.

Roughly 5% of the global population exhibits variations in either the alpha or beta components of the hemoglobin molecule. However, it's important to note that not all of these variations lead to symptoms, and some individuals are classified as silent carriers. In reality, only 1.7% of the worldwide population displays symptoms attributable to gene mutations, a condition referred to as thalassemia trait.

Nonetheless, certain ethnic groups have a higher likelihood of being affected, with prevalence rates of thalassemia symptoms ranging from 5% to 30% within these populations.

Alpha-thalassemia is notably prevalent in specific ethnic groups with Southeast Asian roots. Additionally, there's a substantial presence of carriers in Sub-Saharan Africa and the Western Pacific regions. The distribution of these various population groups varies based on the geographical regions around the world, and it can be outlined as follows:

- **America**: 0-5% of the population has a thalassemia trait, up to 40% of this population possibly being genetic carriers.
- **Eastern Mediterranean**: 0-2% of the population has a thalassemia trait, with up to 60% of this population potentially being genetic carriers.
- **Europe**: 1-2% of the population has a thalassemia trait, with up to 12% of this population being genetic carriers.
- **Southeast Asia**: 1-30% of the population has a thalassemia trait, with up to 40% of this population potentially being genetic carriers.
- **Sub-Saharan Africa:** 0% of the population has a thalassemia trait and up to 50% of this population potentially being genetic carriers.
- **Western Pacific**: 0% of the population has a thalassemia trait, with up to 60% of this population potentially being genetic carriers.

Among populations with Mediterranean, African, and South Asian heritage, beta-thalassemia stands as the most prevalent form of thalassemia. The distribution of this condition among various population groups around the world can be outlined as follows based on geographical regions:

- **America**: 0-3% of the population is affected by a gene mutation
- **Eastern Mediterranean**: 2-18% of the population is affected by a gene mutation
- **Europe**: 0-19% of the population is affected by a gene mutation
- **Southeast Asia**: 0-11% of the population is affected by a gene mutation
- **Sub-Saharan Africa**: 0-12% of the population is affected by a gene mutation
- **Western Pacific**: 0-13% of the population is affected by a gene mutation[3].

Hemoglobinopathies

Hemoglobin (Hb) serves as the molecule responsible for transporting oxygen in red blood cells. In each adult Hb molecule, there are 4 subunits, comprising 2 α-globin and 2 β- (or β-like) globin chains. The α-globin gene cluster is located close to the telomere on the short arm of chromosome 16. On the other hand, the human β-globin spans a region of approximately 70 kb situated on the short arm of chromosome 11 and encompasses five functional genes. Hb A is the dominant form of Hb molecule found in adult humans.

While the α-globin gene cluster experiences a single developmental "switch," the β-gene cluster goes through 2 such transitions. During the embryonic stage, transcription begins with the ε gene but switches to the transcription of the two γ genes after the sixth week of gestation, primarily occurring in the fetal liver and continuing into the prenatal period. Subsequently, this transcription transitions to the δ (minor adult) and β (major adult) genes. Approximately six months after birth, the presence of hemoglobin F (HbF) constitutes less than 5% of the total hemoglobin and gradually decreases, eventually reaching less than 1% in two-year-old individuals.

Inherited hemoglobin disorders constitute extensive groups of autosomal recessive conditions. These disorders arise from over 700 identified defects in globin genes. Autosomal recessive inherited disorders result from either faulty or absent production of one of the globin chains within the Hb tetramer. The specific affected globin chain distinguishes between α-, β-, and δ-thalassemias. The term "thalassemia" originates from the Greek words "thalassa," meaning "sea," and "aemia," indicating "anemia." It was noted that thalassemia is more prevalent in regions where malaria was historically present or endemic.

The underlying pathophysiology of these disorders is rooted in the resultant imbalance in the α:β chain ratio. Both α-thalassemia and β-thalassemia have a notable prevalence in various populations, with β-thalassemia being more widespread and common. β-Thalassemia belongs to a family of inherited hemoglobin disorders characterized by a reduction in the synthesis of β-globin chains. The high frequency of thalassemia can be attributed to the protective advantage it provides against malaria in carriers, similar to the heterozygote advantage

observed in sickle cell hemoglobin carriers. Thalassemias can lead to severe anemia from early in life, and without regular blood transfusions, they can result in death within the first year[2].

Prenatal Diagnosis

The introduction of prenatal diagnosis has provided couples at risk of major hemoglobinopathies with a new avenue and has transformed the approach to screening and counseling for thalassemias. The initial and crucial step in thalassemia prevention involves prenatal diagnosis of these blood disorders. Currently, traditional methods like amniocentesis, chorionic villus sampling (CVS), and cordocentesis are still employed for prenatal thalassemia diagnosis. However, it's important to note that these conventional techniques carry a risk of fetal miscarriage, which is estimated at around 1%.

The American College of Obstetricians and Gynecologists (ACOG) recommends specific steps for managing individuals with certain blood-related conditions:

1. For women exhibiting a low mean corpuscular volume (MCV), ACOG suggests assessing serum ferritin levels. If their levels are normal but they have microcytic anemia, further evaluation through hemoglobin electrophoresis testing is advised.

2. In cases of normal hemoglobin electrophoresis and Asian ancestry, genetic testing for α-thalassemia is recommended. In all these scenarios, it is essential to conduct partner testing to assess the risk of a fetus being affected. Hemoglobin electrophoresis or genetic testing, as appropriate for β- and α-thalassemia, respectively, is utilized for this purpose.

3. Couples identified as carriers of these conditions should undergo genetic counseling to determine the potential risk of having an affected fetus. Additionally, family decision support should be made available to them.

4. The most severe form of α-thalassemia, characterized by the deletion of all four α-genes, results in a condition known as hydrops fetalis. This condition is marked by severe anemia (hemoglobin levels ranging from 3-8g/dL), organ enlargement, and edema. Typically, it leads to fetal demise within the uterus due to heart failure, resulting in preterm labor, stillbirth, and adverse effects on maternal health. Prenatal screening can identify at-risk fetuses, and amniocentesis or chorionic villus sampling can confirm the diagnosis. In suspected cases, Doppler assessments of cerebral vessel flow velocities or direct fetal blood sampling can be utilized to quantify anemia. If intrauterine transfusions are administered promptly, some of these fetuses may survive but would require postnatal transfusion support, classified as α-thalassemia major.

It is important to note that individuals of African ancestry commonly exhibit 2 α-gene deletions; however, these mutations typically occur on different chromosomes (trans configuration). Consequently, the risk of having a fetus with all 4 genes deleted is lower than in individuals with East Asian ancestry, where the 2 gene deletions more frequently occur on the same chromosome (cis configuration).

For women identified as carriers of β-thalassemia trait or HbS trait, partner testing involving hemoglobin fractionation is crucial to assess the fetal risk of β-thalassemia or sickle cell disease.

Before conception, ACOG advises genetic testing and counseling for couples who are at a high risk of thalassemia. For couples who prefer to avoid elective termination, options like in vitro fertilization and preimplantation genetic diagnosis can be considered. In cases where couples were not identified as high risk for thalassemia before pregnancy, the option of DNA testing through chorionic villus sampling or amniocentesis should be presented. Counseling should be offered in all such scenarios. This approach has significantly decreased the incidence of infants born with thalassemia in regions like the Mediterranean, the Middle East, parts of the Indian subcontinent, and Southeast Asia. Ongoing research is exploring innovative methods to reduce the need for invasive procedures like chorionic villus sampling and amniocentesis, including the collection of fetal DNA from fetal cells in maternal blood or plasma.

Ongoing research is focused on non-invasive prenatal detection (NIPD) of α and β thalassemia. The primary challenge in NIPD lies in distinguishing cell-free fetal DNA from maternal DNA. Depending on the parental mutations, 3 primary approaches have been employed to address this challenge: excluding the paternal mutation type, utilizing single-nucleotide polymorphism (SNP) based methods to differentiate the DNA origin, and applying relative mutation dosage (RMD) to identify fetal mutations based on the maternal allele ratio. RMD methods, often utilizing real-time PCR (RT-PCR) to determine the cycle threshold (Ct), which represents the number of PCR cycles required to reach a specific amount of PCR product. In contrast to the traditional Ct approach, this study employed surface-enhanced Raman spectroscopy (SERS) for quantifying thalassemia alleles.

SERS is a scattering spectroscopy technique that detects inelastic vibrations in the secondary structure of molecules, and it possesses the sensitivity to detect even single molecules. This high sensitivity is attributed to plasmonic effects that occur when target molecules adhere to noble metal surfaces. SERS can detect DNA sequences directly or indirectly by measuring Raman-active tags attached to target sequences.

Notably, approximately 3-20% of maternal plasma DNA consists of cffDNA derived from trophoblastic cells, which have shorter sequences than maternal DNA. Consequently, specific amplification and treatment steps are necessary for detecting cffDNA in plasma. Previous approaches have utilized PCR methods for simultaneous detection of three target strands from epizootic pathogens. For mutation detection, methods like mutation-specific PCR and PCR-like techniques such as exponential strand displacement amplification (SDA) and ligase detection reaction (LDR) have been introduced prior to the implementation of SERS. These processes amplify only the mutated sequences and then detect them using SERS. The multiplex PCR method with fluorescence-labeled primers, allows simultaneous amplification of both the SEA mutation and wild-type (WT) alleles. This is particularly useful for comparing the ratios between SEA and WT alleles, and therefore, it was chosen as the pre-treatment method for maternal plasma before SERS measurement[2,4].

Therapy

In the case of minor and minima forms, therapy is typically not necessary. For other forms, regular red blood cell transfusions and possibly the use of iron chelators are indicated based on the clinical situation.

Treatment of α-thalassemias:

- Minima and Minor forms: Usually no therapy is required.
- HbH disease: Intermittent transfusion of red blood cell concentrates may be considered.
- Hb Barts hydrops fetalis syndrome: Usually none, as the decision is often made to terminate the pregnancy or the newborns typically do not survive the perinatal period.

Treatment of β-Thalassemia major:

- Symptomatic treatment options: Symptomatic management of Thalassemia major includes regular red blood cell transfusion therapy combined with chelation therapy to prevent iron overload in the body[1].
- Transfusion of red blood cell concentrates:
  - Procedure: Regular transfusions, typically every 3 weeks, administered alongside combined chelation therapy to avoid secondary iron overload.
  - Initiation of therapy: When repeated hemoglobin (Hb) levels are <8 g/dL or clinically significant symptoms are present.
- Chelation therapy in Thalassemia:
  - Initiation of therapy: Serum ferritin concentrations >1000 µg/L
  - Goal: Prevention and treatment of secondary iron overload.
- Application: Administration of iron chelators as monotherapy or combination therapy, for example, Deferoxamine.

Summary

The identification of individuals with thalassemia syndromes and thalassemia trait has become a crucial health issue. This identification serves the dual purpose of enabling early and comprehensive care while also preventing unnecessary interventions. Early diagnosis is not only essential for preparing at-risk couples but also for identifying fetuses and newborns who may be at risk. Although newborn screening for hemoglobin disorders, initially focused on sickle cell disease, has made significant progress, there is still room for improvement in order to achieve the objective of optimal screening for thalassemia and the establishment of early diagnoses to enhance management strategies[4].

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Literature Review

Congenital Dyserythropoietic Anemia

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Keywords: *Congenital dyserythropoietic anemia, hereditary anemia, iron overload*.

Abbreviations: **CDA** – Congenital Dyserythropoietic Anemia, **CDAN1** – Codanin 1, **CDA-I** – Congenital Dyserythropoiesis Anemia-Type I, **CDA-II** – Congenital Dyserythropoiesis Anemia-Type II, **XLTDA** – X-linked recessive hematologic disorder, **DNA** – Deoxyribonucleic Acid, **GATA1 – GATA** Binding Protein 1, **LPIN2** – Lipin 2, **VUS** – Variant of Uncertain Significance, **NGS** – Next Generations Sequencing, **CBC** – Complete Blood Count, **G-6PD** – Glucose-6 Phosphate Dehydrogenase, **NSAID** – Non-steroidal anti-inflammatory drugs, **RBC** – Red Blood Cells.

Abstract

Congenital dyserythropoietic anemia (CDA) is a rare inherited hematological disorder characterized by impaired erythroblast maturation, leading to anemia. An overview of CDA with an emphasis on its molecular underpinnings, clinical characteristics and manifestations are provided in this article.

Mutations in crucial genes for erythroid differentiation, which affect nuclear morphology and hemoglobin production, are part of the physiopathology of CDA. Jaundice, hepatosplenomegaly and anemia are a few examples of the clinical symptoms. Genetic test and bone marrow analysis are used for diagnosis.

1. Introduction

Congenital dyserythropoietic anemia (CDA) is one of a heterogenous group of inherited anemias characterized by ineffective erythropoiesis[1]. Consequently, this disorder affects the evolution of erythrocytes. This illness is one of numerous varieties of anemia, a condition marked by a deficiency in red blood cells. Due to this deficiency, the blood is unable to provide enough oxygen to the body's tissues[2].

2. Pathogenic mechanism

Based on their unique structure, clinical and hereditary characteristics, CDA's are divided into three primary kinds (I, II, III), as well as transcription factor-related CDA's and CDA variants.

CDA-I is caused by bi-allelic mutations in either *CDAN1* or *C15orf41* and other 56 causative mutations that has been discovered[3].

It is probable that both of the proteins that these two genes expression will play crucial roles in DNA repair and/or chromatin reassembly after DNA replication.

The *C15orf41* protein is predominantly localized either to the cytosol or to the nucleus. This implies that this protein has a dual role inside these two subcellular domains[4].

3. Clinical manifestations

Majeed syndrome is an extremely rare autosomal recessive ailment that is characterized by decreasing in erythrocyte color and in size than normal with dyserythropoiesis, inflammatory dermatosis and persistent recurrent multifocal osteomyelitis.

The causative gene is *LPIN2*, which encodes a phosphatidate phosphatase, important in lipid metabolism[5].

As for other types of hereditary anemias, the classical diagnostic workflow for CDA includes different lines of examination, which start from the analysis of family and personal history, move on to biochemical and structural evaluation and end with genetic testing.

The next following items are clinical manifestations for CDA[6]:

- Typically manifesting in childhood, chronic moderate congenital anemia (red cells with nonspecific abnormalities, basophilic stippling, sporadic normoblasts).
- Given the extent of anemia and the presence of increased count of erythroid precursor cells in the bone marrow, the reticulocyte response in inadequate.
- Granulopoiesis and thrombopoiesis in a normal state.
- Moderate jaundice that is persistent or sporadic.
- Splenomegaly.
- Hemosiderosis is caused by high rate of plasma iron turnover and a poor rate of erythrocyte iron utilization (ineffective erythropoiesis).
- Shortened red cell survival time.
- Hemosiderosis from progressive iron overload.
- Marrow that generally distinguishes between the three forms of CDA due to aberrant erythroid morphology.

4. Clinical results

The results show that treating CDA-I patients holistically and cooperatively produces the greatest outcomes. This is in contrast to the careful treatment of iron excess and the use of interferon alpha.

The study of iron metabolism in CDA-II has made the most strides in our understanding of CDA's. It has been shown that the erythroblast-produced hormone erythroferrone mediates hepatic iron overload by specifically blocking the production of hepcidin.

CDA-III is the most unique of these three classical CDA forms[7].

A set of clinically diverse disorders with bleeding propensity, minor to major type of anemia and abnormal large platelets with over granulated platelets make up XLTDA. Severe bleeding and/or blood transfusion dependency occur at the clinically most severe end of the spectrum and last a lifetime. Dysfunctional red blood cell development and impaired megakaryopoiesis and characteristics of the bone marrow with ageing.

Mutations in GATA1, a X-linked gene (the X-linked gene it describes features or traits that are impacted by genes on the X chromosome) that codes for a DNA-binding protein with two zinc bonds and a transactivation domain, lead to XLTDA. GATA1 has a necessary role in evolution and sustention of both erythroid and megakaryocytic lineages. Considering the X-linked patrimony, males are primarily impacted, and the severity and specificity of the phenotype depends on the imbalance in GATA1 function[8].

We can discuss the difference between patients with transfusion-dependent anemia (TD) and the patients with non-transfusion-dependent anemia (NTD).

For the TD patients, the principal steps to reach molecular diagnosis of these conditions are: NGS-based genetic testing (pathogenic variants in CDA related genes, VUS in CDA related genes, pathogenic variants or VUS in genes related to other anemias) and single/multiple gene testing.

NGS-based hereditary testing speak to an enormous parallel sequencing technology

that gives ultra-high throughput and speed. This innovation is used to decide the arrange of nucleotides in whole genomes or focused on locales regions of DNA or RNA[9].

For the NTD patients, by testing CBC investigation and particular tests.

Table 1. Investigations and particular tests for each type of CDA.

CDA type	Investigations that need to be done
CDA-I	Examination of skeletal mutations.
CDA-II	Examination of bone marrow; Investigation of 3-hypoglycosylation by sodium dodecyl sulphate polyacrylamide gel electrophoresis.

5. Complications

The main CDA problems associated with chronic hemolytic anemia (lower number of erythrocytes due to hemolysis) include iron excess, edema in an

unborn or newborn baby, aplastic crisis, hyperbilirubinemia, gallstones and enlarged spleen. A common problem with CDA's is iron overload[10].

In correlation with what has been said above, there are some complications and modern therapeutic elucidations.

Table 2. Current therapeutic solutions in CDA problems and complications.

CDA manifestation	Complications	Current therapeutic solutions
Iron overload[11]	Liver damage; Liver cirrhosis; Injury to the islet cells of the pancreas; Diabetes; Hypothyroidism; Hypogonadism.	Reduction therapy; Blood transfusion; Iron chelation therapy;
Hydrops fetalis[12]	Fluid around the heart and lungs; Severe low blood sugar; Lungs development issues (underdeveloped); Severe anemia.	Fetal blood transfusion; Early caesarean delivery; Giving blood to the baby while still in the womb.
Aplastic anemia[13]	Bleeding; Infections.	Ticlopidine; NSAID; Immunosuppressive therapy using: eltrombopag; horse/rabbit anti-thymocyte globuline, cyclosporine A.
Hyperbilirubinemia[14]	Brain and spinal cord injuries.	Intravenous immunoglobulin; Exchange transfusion; Cholestyramine.
Enlarged spleen[15]	Splenic rupture; Cytopenias; Low number of RBC, leukocytes and platelets.	Antibiotics; Splenectomy; Blood transfusions; Exchange transfusions.
Gallstones[16]	Acute cholecystitis; Jaundice; Infection of the bile ducts; Acute pancreatitis.	Ursodiol link and chenodiol link; Cholecystectomy.

The mechanism of hepatic iron excess has been studied recently, primarily for CDA-II. Hepcidin's expression was previously shown to be reduced, and other erythroid regulators, such as growth/ differentiation factor 15, have been suggested as pathological suppressors of hepcidin expression[17].

The differential diagnosis incorporates other haemolytic anemias, such as: congenital spherocytic anemia, paroxysmal nighttime hemoglobinuria, chronic non-spherocytic haemolytic anemia due to G-6PD insufficiency, autoimmune haemolytic anemia and anemias with ineffectual erythropoiesis like thalassemia and myelodysplastic disorders.

Inherent spherocytic anemia – the foremost common misdiagnosis – is effortlessly recognized by the nonattendance of anisopoikilocytosis (variation of shape and size of red blood cells) and the higher rate of reticulocytes, in advance of more particular tests, such as osmotic delicacy and protein electrophoresis[18].

6. Conclusions

The morphological characteristics of CDA erythroblasts point to their delayed maturation into adult erythrocytes. Even though a number of CDA associated genes have been recognized, it is frequently unclear how their modified functions contribute to erythroblast multinuclearity. Nevertheless, research on the pathogenic mechanisms underlying CDA's continues to yield new findings that are helpful for the clinical management of these patients as well as the investigation of novel therapeutic approaches.

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Literature Review

Hemophilia C in Women

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Keywords: Factor XI, Rosenthal syndrome, prolonged bleeding, mutation, fresh frozen plasma FFP, desmopressin, pregnancy, menorrhagia.

Abstract

Factor XI (FXI) deficiency (hemophilia C or Rosenthal syndrome) is a genetically-transmitted disease usually inherited in an autosomal recessive pattern associated with genetic defects in the FXI gene. It affects both genders equally, but studies show that the disorder is more common in Ashkenazi Jews. People with hemophilia C do not have spontaneous bleeding, symptoms like uncontrollable or prolonged bleeding, epistaxis, hematuria or bruising occur after serious injuries, major surgeries or after giving birth. In female it may cause menorrhagia. Unless there's an undergoing surgery or an injury that may cause severe bleeding, people with hemophilia C do not require treatment. Fresh frozen plasma, anticoagulant medication or birth control might reduce the risk of excessive bleeding.[1]

Introduction and etiology

Factor XI (FXI), also known as plasma thromboplastin antecedent (PTA), is a key player in the intrinsic pathway of the coagulation cascade. It acts by activating FIX, which, in turn, contributes to the generation of thrombin and the formation of a stable blood clot.

Factor XI is a dimeric serine protease, which is composed of chains that each weigh 80,000 Da. Factor XIIa activates factor XI and factor IX in the original intrinsic pathway of blood coagulation. Also, thrombin directly activates factor XI, and this direct activation may be more important than the activation due to factor XII. Recently, it has been shown that thrombin activation of factor XI is triggered by polyphosphate release from activated platelets. These molecules provide a template for assembly of factor XI and factor IX. Patients with factor XII deficiency, even severe deficiency, do not necessarily have a tendency to bleed. Therefore, the absence of factor XII appears to be irrelevant to factor XI.

Factor XI is a zymogen that, on activation, undergoes conversion to a serine protease that leads to activation of factor IX, followed by thrombin generation. The sustained generation of thrombin also leads to the activation of thrombin-activatable fibrinolysis inhibitor (TAFI), which impairs the conversion of plasminogen to plasmin. Thus, factor XI serves as a procoagulant and an antifibrinolytic agent, and the lack of factor XI in plasma results in a tendency to bleed. People with severe factor XI deficiency have a lower incidence of ischemic stroke.[2]

Factor XI does not play a role in the complement or kinin pathways, however, it has been demonstrated to contribute to the activation of fibrinolysis. The primary inhibitor of factor XIa is alpha-1 antitrypsin, responsible for approximately two-thirds of its inhibition. The remaining inhibition is attributed to C1 esterase inhibitor, antithrombin III, and alpha-2 antiplasmin.[2]

In individuals with a significant deficiency of factor XI, bleeding typically occurs in response to injury, particularly when trauma involves tissues abundant in fibrinolytic activators, such as the oral mucosa, nasal tissues, or the urinary tract. Unlike individuals with severe hemophilia A or B, those with a major deficiency of factor XI do not experience spontaneous bleeding events.

Factor XI deficiency is an uncommon autosomal bleeding disorder linked to genetic anomalies within the FXI gene. The gene for factor XI is near the gene for prekalikrein on the distal arm of chromosome 4 (4q35). It is 23 kb, with 15 exons and 14 introns. Factor XI is synthesized in the liver and circulates in the plasma as a complex with high-molecular-weight kininogen. Factor XI has a half-life of about 52 hours.[2] This condition exhibits significant heterogeneity, manifesting as varying tendencies towards bleeding and diverse causal mutations within the FXI gene. It can be classified into two main categories: cross-reacting material-negative (CRM-) FXI deficiency, characterized by reduced FXI levels, or cross-reacting material-positive (CRM+) FXI deficiency, marked by impaired FXI function. The FXI mutation database has reported an increasing number of mutations, primarily impacting the serine protease (SP) domain of the FXI protein. The functional analysis of these mutations contributes to a deeper understanding of the molecular mechanisms underpinning FXI deficiency.[3]

Epidemiology

Hemophilia C is more common in certain populations and geographic regions. It has a higher prevalence among Ashkenazi Jews of Eastern European descent, living in Israel, with an estimated frequency of 8-9% of Ashkenazi Jews being carriers of the genetic mutation that causes factor XI deficiency. Two mutations are predominant in this group: type II (Glu117Stop), about 30% of homozygous patients develop inhibitors for FXI and type III (Phe283Leu).[4] In other populations, the prevalence is generally lower, affecting 1out of 100.000 people worldwide. Both genders are equally affected.[5] Moreover, people of any age group can be affected. To note is the fact that normal infants younger than age 6 months have low levels of factor XI because of the time required for factor XI to reach normal levels observed in adults. After this is reached, factor XI levels do not change with age.[2]

Diagnosis

It is not easy to diagnose hemophilia C. The genetic analysis could be helpful to determine which mutation caused the factor XI deficiency. Imaging studies bring no further information about the pathology unless, there is an actual bleeding happening, it might help to evaluate the extent of the bleeding. Most important for the diagnosis are the laboratory studies which include: complete blood count (CBC), measurement of factor XI levels, measurement of factor VIII and von Willebrand factor, prothrombin time (PT), thrombin time (TT) which are normal and activated partial thromboplastin time (aPTT) which is usually prolonged. Rosenthal Syndrome may coexist with other deficiencies so as assays of other clotting factors and platelet function may be needed. The deficiency is categorized as major when the factor XI levels are 15-20 U/dl or lower in patients with at least 2 FXI gene mutations. Individuals with partial deficiency, generally heterozygotes with a single FXI gene mutation, have levels of 20-60 U/dl. Unlike other coagulation problems, the severity and chance of bleeding correlates poorly with factor levels.[2]

Differential diagnosis

The diagnosis of Hemophilia C is primarily established through laboratory testing, which involves demonstrating insufficient levels of factor XI activity. While other bleeding disorders may present with similar clinical symptoms, the differentiation between these conditions is largely reliant on laboratory results. Congenital hemophilia A and B are clinically similar but unlike hemophilia C these conditions are distinguished by the occurrence of deep and spontaneous bleeding episodes. A similar clinical picture has the von Willebrand disease, which results from deficiency or abnormal function of this factor, which role is to initiate platelet adhesion in primary clot formation and also to stabilize factor VIII. Any other coagulation factor deficiency may present with a variety of bleeding patterns, similar to the Rosenthal syndrome.[6]

Treatment and management

Because the severity of bleeding cannot be predicted by the levels of factor XI in the blood, the treatment and management of hemophilia C could be questionable. However, many options have been found to prevent the excessive bleeding. As the highpoint of this article is the FXI deficiency in

women, treatment and management options will be discussed in correlation with gynecological procedures and surgeries.

Prophylactic therapy is advised for women with severe factor XI deficiency undergoing surgery. However, the guidance for heterozygotes with mildly reduced activity levels and no prior surgical history is less straightforward. Studies show that individuals with further factor deficiencies like low von Willebrand factor, thrombomodulin and von Willebrand ristocetin cofactor were more likely to bleed during surgical procedures and were categorized as "bleeding" individuals.[7,8] Another study explored thrombin generation in two groups: one consisting of 9 individuals with a documented history of bleeding and the other comprising 15 subjects without any bleeding episodes during previous traumatic events or surgical procedures. Regardless of their factor XI activity levels, individuals with a history of bleeding exhibited significantly reduced *in vitro* thrombin generation characteristics. These included extended lag time, reduced peak thrombin levels, and diminished thrombin generation velocity. These values were lower than those observed in normal control subjects and factor XI-deficient patients who did not have a history of clinical bleeding. This raises the possibility of utilizing thrombin-generation assays as a predictive tool for assessing bleeding risk.[7,9]

The most common management when it comes to factor XI deficiency-related bleeding is the **fresh frozen plasma FFP**, which may be used also as prophylaxis. The daily administration dose is about 20 ml plasma/kg, with a factor goal between 20 and 50%, depending on the nature of the procedure. Potential side effects of FFP transfusion include transfusion-associated circulatory overload, transfusion reaction, alloimmunization, anaphylaxis, and transfusion-related acute lung injury.[7]

Desmopressin, which is a synthetic analog of vasopressin, is used as a hemostatic agent in various bleeding disorders. These include conditions such as von Willebrand disease, factor VIII deficiency, functional platelet disorders, and bleeding secondary from uremia. The mechanism of action of desmopressin involves its binding to the vasopressin V2 receptor located on vascular endothelial cells. This interaction leads to the release of von Willebrand factor, consequently enhancing hemostasis by elevating both von Willebrand factor and factor VIII levels. It has been used in one study for minor procedures such as hydrocele repair or reconstruction the urethra in individuals with minor FXI deficiency. The dose administrated was 0,3 μg/kg for 5 days starting on the day of the surgery and no complications occurred.[7,10,11] Some side effects are headaches, flushing, fluid retention and hyponatremia.

Antifibrinolytic drugs include **TXA** and **epsilon aminocaproic acid**. These lysine analogs bind to the lysine-binding site of plasminogen preventing the binding to fibrin and reducing the risk of postpartum hemorrhage.[7] Side effects are not so usual, some of the most common are abdominal or stomach pain, chills or fever, headache.

The administration of **recombinant factor VIIa** has been used in major surgical procedures in patients with severe FXI deficiency, who already have inhibitors for FXI or patients who are at high risk of developing them.[6]

Another therapy option is the usage of **plasma-derived FXI concentrate**. It is known under the name of Hemoleven, contains AT, heparin and C1 esterase inhibitor and was developed in France and available there since 1992. Thrombotic events are a risk in some patients receiving this treatment, especially those with preexisting risk factors such as older age, peripheral or central vascular disease, morbid obesity. There's been studies that show people developing pulmonary embolism and transient ischemic attacks after the treatment with Hemoleven. The ratio between risk and benefit is still questionable.[12,13]

Pregnancy and FXI deficiency

Pregnant individuals with factor XI deficiency are also at risk of bleeding complications during childbirth. However, this scenario is influenced by the hypercoagulable state that typically accompanies pregnancy, leading to elevated levels of factors V, VII, VIII, IX, and X. In contrast, there is no consistent alteration in factor XI levels during pregnancy. While one study indicated an increase in factor XI levels, other researchers have reported a mild decrease in these levels. Other studies show no correlation between the severity of bleeding during childbirth and the levels of FXI in blood. The best predictor was the outcome during a previous pregnancy and prophylaxis was not mandatory for vaginal delivery.[14,15]

Menorrhagia in FXI deficiency

The hemostatic system plays a pivotal role in regulating both the quantity and duration of menstrual bleeding. Consequently, women who have inherited bleeding disorders often experience

abnormally prolonged or heavy menstrual bleeding. It's worth noting that irregular, premenarchal, or postmenopausal uterine bleeding is atypical in individuals with inherited or acquired hemorrhagic disorders. Instead, common manifestations in these individuals include severe acute bleeding and heavy menstrual bleeding at the time of menarche and persistent, chronic menorrhagia throughout their reproductive years. A comprehensive, multidisciplinary approach is essential for the diagnosis and treatment of individuals with bleeding disorders and associated menorrhagia. Treatment options for menorrhagia in these cases are generally in line with those used for menorrhagia in the general population, with some additional considerations, including desmopressin and replacement therapy, while non-steroidal anti-inflammatory drugs should be avoided. The choice of treatment should take into account various factors, including the patient's preferences, age, and the severity of bleeding. Iron supplementation is very important to address potential iron-deficiency anemia. Initial remedies commonly used for menorrhagia, such as tranexamic acid, combined oral contraceptives, and the levonorgestrel intrauterine system, are typically attempted first. In cases of treatment failure or contraindications, before considering surgical options, treatment with desmopressin is often the preferred choice, especially in patients known to be responsive to it. The availability of desmopressin preparations for self-administration has made home treatment a viable option in carefully selected cases. This form of treatment has proven to be effective and safe when patients are educated to self-administer the medication exclusively during the first two or three heaviest days of their menstrual period. This self-administration should be limited to a maximum of three to four doses, with no more than two consecutive administrations at a 12-hour interval.[16]

Conclusion

In conclusion, factor XI deficiency is a rare autosomal recessive bleeding disorder, most prevalent among Ashkenazi Jews. Spontaneous bleeding episodes are infrequent, and the risk of postoperative bleeding varies widely and doesn't consistently correlate with factor XI activity levels. This discrepancy is particularly evident in gynecological patients.

Prophylactic treatment options are determined based on the extent of surgical invasiveness and may include fresh frozen plasma (FFP), antifibrinolytics, desmopressin, and recombinant factor VIIa. The choice of prophylaxis depends on the specific surgical situation and the patient's individual needs.

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Literature Review

Therapeutic Trends and Perspectives in Diamond Blackfan Anemia

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Keywords:
Diamond Blackfan anemia, *bone marrow failure*, *blood transfusion*, *hematopoietic stem cell transplantation*, *gene therapy, genome editing.*

Abbreviations:
DBA – Diamond Blackfan anemia, **DBAR** – Diamond Blackfan Anemia Registry of North America, **HbF** – fetal hemoglobin, **eADA** – erythrocyte adenosine deaminase, **CHH-AD** – cartilage hair hypoplasia - anauxetic dysplasia, **TCS** – Tracher Collins syndrome, **PRCA** – pure red cell aplasia, **RPS** – ribosomal protein small subunit, **RPL** – ribosomal protein large subunit, **AML** – acute myeloid leukemia, **MDS** – myelodysplastic syndrome, **IBMF** – inherited bone marrow failure, **HSCT** – hematopoietic stem cell transplantation, **RBC** – red blood cells, **GCs** – glucocorticoids, **GCR** – glucocorticoid receptor, **DFX** – Deferasirox, **DFO** – Deferoxamine, **DFP** – Deferiprone, BFU-E – burst forming unit-erythroid, **CFU-E** – colony forming unit-erythroid, **cGvHD** – chronic grafts versus host disease, **aGvHD** – acute grafts versus host disease, **HLA** – human leukocyte antigen, **AIEOP** – Italian Association of Paediatric Haematology and Oncology Registry, LVs – lentiviral vectors, **EFS** – elongation factor 1α short, **ZFNs** – Zinc finger nucleases, **TALENs** – transcription activator-like effector nucleases, **CRISPR/Cas9** – clustered regularly interspaced short palindromic repeats-associated Cas9, **mTOR** – mammalian target of rapamycin, **SMER** – small molecule enhancers of rapamycin.

Abstract

Blackfan Diamond anemia is a rare clinical entity caused by mutations in genes encoding ribosomal proteins.

Erythrogenesis defects, somatic malformations, and increased risk of developing hematological malignancies or solid tumors place the affected pediatric population in the focus of specialists.

While classical approaches such as blood transfusions and stem cell transplantation aim to relieve anemia and correct bone marrow dysfunction, therapeutic strategies based on *L-Leucine*, *Sotatercept*, *Trifluoperazine*, *SMER28*, and *Eltrombopag* seek to improve the production of erythroid precursors.

Even if in its nascent stages, therapeutic directions focused on processing genetic material aim to remedy abnormal gene expression.

This literature review aims to highlight treatments used to improve blood-forming function and therapeutic prospects that could increase the life expectancy of patients with Diamond Blackfan anemia.

Introduction

Blackfan Diamond Anemia (DBA) is an inherited bone marrow failure syndrome characterized by deficient production of erythroid precursors in association with craniofacial, upper limb, genitourinary, and cardiac malformations.

According to studies conducted by Diamond Blackfan Anemia Registry of North America (DBAR), half of patients are diagnosed within the first 3 months of life, while 90% of cases are identified by the age of one year.[1]

DBA usually manifests as a macrocytic and a regenerative anemia.

Even if other cell lines are normal, some patients may present with neutropenia, thrombocytopenia, or thrombocytosis.

Patients often have increased fetal hemoglobin (HbF) and increased erythrocyte adenosine deaminase activity (eADA).[2]

The clinical picture of DBA includes pallor, growth disorders[3], and multiple congenital malformations such as microcephaly, micrognathia, microtia, epicanthus, palatoschisis, short neck, absence of kidney, horseshoe kidney, hypospadias, coarctation of the aorta, tetralogy of Fallot.

Some patients may also see absent, hypoplastic, bifid, or triphalangeal thumb and flat thenar eminence.[1]

Differential diagnosis may include a multitude of syndromes that sum up a range of organic or limb malformations such as Fanconi Anemia, Shwachman-Diamond syndrome, Pearson syndrome, dyskeratosis congenita[1], cartilage-hair hypoplasia-anauxetic dysplasia (CHH-AD) and Treacher Collins syndrome (TCS).[4]

DBA is included in the category of rare diseases, being estimated between 1 and 4 cases /500,000 live births in a year, similarly affecting both sexes and different racial groups.[5]

According to studies, DBA is a form of congenital pure red cell aplasia (PRCA)[6], resulting from monoallelic mutations in ribosomal genes: RPS7, RPS10, RPS17, RPS19, RPS24, RPS26, RPL5, RPL11, RPL26, RPL35A.

Although the most common mutation detected appears to be in the RPS19 gene, there are families whose mutant genes also include mutations in RPL3, RPL7, RPL9, RPL14, RPL18, RPL19, RPL23A, RPL26, RPL35, RPL36, RPS8, RPS15, RPS27A.[7]

Beyond ribosomal gene defects, in some cases, the disease can be caused by mutations in GATA1, also called erythroid transcription factor.

This transcription factor is required for normal hematopoiesis.[5,8]

Although DBA is inherited in an autosomal dominant manner in 40-50% of cases, recent studies do not rule out the possibility that the disease occurs independently of family history.[5]

Cases of DBA require increased attention from clinicians because this syndrome is associated with a predisposition to developing malignancies such as acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), acute lymphoblastic leukemia, Hodgkin's and non-Hodgkin's lymphoma, breast cancer and hepatocellular carcinoma.[9]

As opposed to other inherited bone marrow failure (IBMF), DBA predisposes to the development of solid tumors such as osteosarcoma, colon cancer, and lung cancer.[10]

Materials and methods

Reviews, clinical cases, and studies relevant to the topic from specialized sources (PubMed, Wiley Online Library, MPDI, Elsevier) were used for this article.

The information presented was selected from documents published between 2016-2022, except for three publications published in 2008, 2010 and 2014.

The keywords used for the search were "Diamond Blackfan anemia", "therapy" and "treatment".

For the description of therapeutic strategies have been included papers exposing the benefits of therapy, adverse reactions, risks, and their complications.

Papers with unavailable full text and those dealing with outdated therapies or without notable benefits were excluded.

Current therapeutic methods

First-line therapy includes blood transfusions, corticotherapy, and hematopoietic stem cell transplantation (HSCT).

Given the deficient production of erythroid precursors, blood transfusions are an effective choice for correcting anemia.

Generally, clinicians aim to maintain hemoglobin levels above 80g/l by administering 10-15 ml/kg red blood cells (RBC) every 3-5 weeks.

To promote growth and development, it is especially important to keep hemoglobin levels over 90 g/l in newborns and young children.
Beyond trying to treat anemia, transfusion therapy is closely related to corticosteroid therapy.

Transfusions are the best method to treat patients under one year of age who do not respond to glucocorticoid therapy or who need higher doses of the drug, while also avoiding the side effects of glucocorticoids.[3]

The establishment of chronic transfusion therapy requires prophylaxis of iron overload.

In this regard, oral or parenteral administration of chelators such as *deferasirox* (DFX) or *deferoxamine*

(DFO) is of benefit to patients with repeated transfusions.

Unlike other congenital anemias, patients with DBA develop secondary hemochromatosis more rapidly, often associated with cardiac complications through the deposition of large amounts of iron in the heart muscle, which predisposes them to early heart failure.[11]

Even if *deferiprone* (DFP) is an effective chelator in removing excess cardiac localized iron, its induced agranulocytosis makes its use in DBA difficult.[11,12]

According to DBAR studies, an increased percentage of patients respond to *prednisone* or *prednisolone* therapy.

While complete blood count and reticulocyte count are monitored weekly, maintaining hemoglobin values above 9 g/dl in the absence of transfusions proves the effectiveness of treatment.[1]

Administration of glucocorticoids (GCs) activates glucocorticoid receptors (GCRs) and causes transcription of *Myb*, *Kit*, and *Lmo2* factors, involved in the proliferation of burst-forming unit-erythroid (BFU-E) and colony-forming unit-erythroid (CFU-E).[13]

It is important to note that during treatment, patients are prone to infections with opportunistic bacteria, such as *Pneumocystis jirovecii*, which requires prophylactic administration of *Sulfamethoxazole-trimethoprim.*[1]

During long-term cortisone therapy, side effects such as demineralization and bone fractures, osteoporosis, hypertension, diabetes mellitus, glaucoma, cataracts, vascular necrosis, and growth deficiency are highly possible, probable, and of increased intensity.[1,14]

Nowadays, hematopoietic stem cell transplantation (HSCT) is a therapeutic method that offers a chance to cure patients with DBA.

At the same time, patients who develop myelodysplastic syndromes (MDS) secondary to DBA, resistant to steroids, and dependent on transfusions can obtain improvement in the quality of life after HSCT.[15]

Recent studies show that stem cell transplantation should be performed before the patient is 10 years old because post-transfusional iron overload is associated with the development of chronic graft-versus-host disease (cGvHD).[16]

Even if the cells can be taken from a donor unrelated to the patient, an HLA-matched sibling without genetic mutations or specific DBA manifestations is the best option.

If the donor is not related to the patient, the transplant can be carried out provided the marrow is a perfect match.

About the source of stem cells, studies show that stem cell collection from bone marrow has a lower risk of cGvHD compared to cells from peripheral blood.[3]

Cord blood is also a source of stem cells associated with satisfactory results in related donors.[15]

According to studies conducted by DBAR and the Italian Association of Paediatric Haematology and Oncology Registry (AIEOP), total body irradiation used in unrelated donor HSCT and *busulfan*-based conditioning therapy used in related donor HSCT caused osteosarcoma.

In this regard, *fludarabine* and *treosulfan* therapy, an agent with lower hematological toxicity, has been tried for a lower intensity of the preparative regimen.[17]

In opposition to the excellent results of HSCT are the risk of acute and chronic graft versus host disease, infertility, and death caused by multiple viral infections.[15]

Alternative therapies

According to a multicenter study by A. Vlachos, supplementation of *L-Leucine* increased reticulocyte counts and improved growth deficits in children with DBA.[18]

While a phase II study expresses the efficacy of *Sotatercept (ACE-011)* in correcting chemotherapy-induced anemia, another trial conducted by A. Vlachos supports the safety of its administration in DBA patients.[19,20]

According to a recent study, administration of *Eltrombopag* to patients with DBA resulted in improved red blood cell production due to chelating properties and the ability to correct disorders of heme and globin synthesis.[21]
In addition, recent papers mention the possibility of preventing pancytopenia in elderly patients treated with *Eltrombopag.*[22]

Furthermore, DBA patients treated with *trifluoperazine*, a calmodulin inhibitor, experienced better erythroid differentiation and decreased p53 protein activity.[23]

Designed as a mechanistic target of rapamycin (mTOR), *SMER28* is an autophagy-inducing

molecule used to ameliorate neurodegenerative processes.

In DBA patients, the introduction of *SMER28* into the therapeutic plan resulted in the multiplication of erythrocytes, due to its action on immature erythroid precursors and erythroblasts.

Last but not least, *SMER28* was able to correct deficient globin and heme expression caused by the RPS19 gene defect.[24]

Strategies based on genetic material processing

Gene therapy

Because 25% of patients have the RPS19 gene mutation, gene therapy is an attractive treatment option.[25]

This approach aims to proliferate erythroid colonies by transferring cDNA into CD34+ cells using lentiviral (LVs) or oncoretroviral vectors.[26]

Although bone marrow failure could be treated with lentiviral vectors, therapy does not exclude the risk of insertional mutagenesis.

In a study conducted on a RPS19-deficient mouse model, the use of the EFS-RPS19 vector caused increased expression of mRNA RPS-19 and contributed to the differentiation of erythroid precursors, without generating hematological abnormalities and increased the risk of insertion mutagenesis.[25,27]

Genome editing

By correcting aberrant genetic sequences, editing the human genome offers the chance to treat congenital or acquired disorders and allows the management of hematological diseases.[28]

Among ZFNs, TALENs, and CRISPR/Cas9, the most commonly used genome editing technologies, CRISPR/Cas9 is the most recent and promising method.

Since the DNA repair system in DBA is unaffected, CRISPR/Cas9 technology should be more effective than in diseases associated with DNA repair deficiency.[26]

Although the risk of oncogenesis is lower than with lentiviral vector gene transfer, off-target nuclease activity can disrupt the response of genes involved in tumor suppression.[28]

Discussions

Although DBA is listed as a rare disease, further research into early diagnosis and curative treatment without major side effects is extremely important.

Even though the life expectancy of these children has increased significantly, it is very important to have a well-established program of screening for potential oncological abnormalities at risk of developing as well as adverse reactions due to chronic medication.

Also, within the family, members should be advised to consult a geneticist for genetic advice.

Because the incidence of occurrence of the disease is extremely low, the diagnosis of DBA is often missed when the clinician is faced with an anemic child with various associated congenital malformations, mainly because other differential diagnoses are more likely and more common.

Conclusions

Research findings on DBA highlight that the management of these patients remains a challenge for clinicians.

Beyond the favorable prognosis of the disease, complications from conventional therapies can alter the quality of life, while the risk of neoplasia contributes to reduced life expectancy.

Although alternative therapies and strategies based on the processing of genetic material seem to have a promising future, further research is needed into their feasibility as a curative treatment for DBA.

Author contributions

The author contributed to the reviewing of the previous literature on the subject, the collection of data from specialized sources and the drafting of the article.

Conflict of interest

The author declares that there are no conflicts of interest concerning this article.

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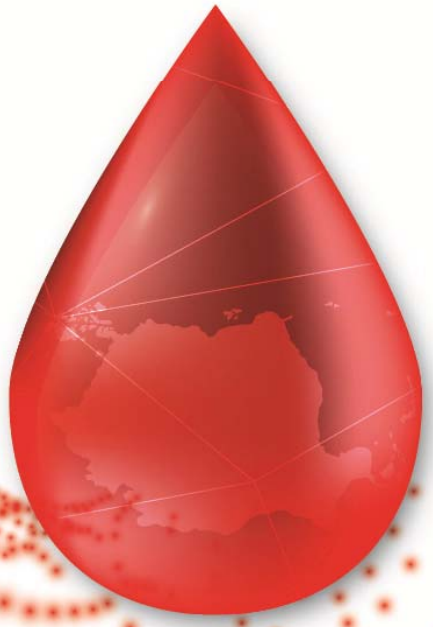

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13:00-13:15	**CEREMONIA DE DESCHIDERE A CONFERINȚEI**
13:15-14:30	**SESIUNEA URGENȚE HEMORAGICE ȘI TROMBOTICE ÎN HEMOPATIILE MALIGNE ȘI PERIOPERATOR**
14:30-14:45	**DISCUȚII ȘI CONCLUZII**
14:45-15:00	**Pauză de cafea**
15:00-16:15	**SESIUNEA DE LA TEORIE LA PRACTICĂ – HEMOSTAZA ÎN MEDICINA DE URGENȚĂ**
16:15-16:30	**DISCUȚII ȘI CONCLUZII**
16:30-17:50	**SESIUNEA PATOLOGIA HEMOSTAZEI ÎN CARDIOLOGIE** **Partea I – Probleme practice în terapia antitrombotică la pacientul cu ateroscleroză**
17:50-18:00	**CONCLUZII ȘI DISCUȚII**
18:00-18:20	**Pauză de cafea**
18:20-19:40	**SESIUNEA PATOLOGIA HEMOSTAZEI ÎN CARDIOLOGIE** **Partea II - Probleme practice în tratamentul anticoagulant**
19:40-20:00	**CONCLUZII ȘI DISCUȚII**
20:00	**CINĂ**

VINERI, 10 NOIEMBRIE 2023

SALA PARIS

08:00-09:20	**SESIUNEA PATOLOGIA HEMOSTAZEI ÎN PEDIATRIE – PARTEA I**
09:20-09:35	**DISCUȚII ȘI CONCLUZII**
09:35-09:50	**SIMPOZION ROCHE**
09:50-10:00	**Pauză de cafea**
10:00-11:40	**SESIUNEA PATOLOGIA HEMOSTAZEI ÎN PEDIATRIE – PARTEA II**

11:40-12:00	**Pauză de cafea**
12:00-13:00	**MASĂ ROTUNDĂ – ACTUALITĂȚI ÎN TESTAREA DE LABORATOR A HEMOSTAZEI**
13:00-13:15	**CONCLUZII ȘI DISCUȚII**
13:15-13:45	**SIMPOZION TOP DIAGNOSTIC**
13:45-14:05	**SIMPOZION DIAMEDIX**
14:05-15:00	**Pauză de prânz**
15:00-16:30	**SESIUNEA PROGRESE ÎN TROMBOCITOPENIA IMUNĂ ȘI PURPURA TROMBOTICĂ TROMBOCITOPENICĂ**
16:30-16:50	**CONCLUZII ȘI DISCUȚII**
16:50-17:10	**SIMPOZION SANOFI**
17:10-17:30	**SIMPOZION MEDIST**
17:30-17:45	**Pauză de cafea**
17:45-19:25	**SESIUNEA PATOLOGIA TROMBO-HEMORAGICĂ ÎN NEOPLAZIILE HEMATOLOGICE**
19:25-19:45	**CONCLUZII ȘI DISCUȚII**
19:45	**CINĂ**

SÂMBĂTĂ, 11 NOIEMBRIE 2023

SALA PARIS

09:00-10:20	**SESIUNEA SINDROMUL ANTIFOSFOLIPIDIC ÎN 2023 – PARTEA I**
10:20-10:40	**DISCUȚII ȘI CONCLUZII**
10:40-11:00	**Pauză de cafea**
11:00-12:20	**SESIUNEA SINDROMUL ANTIFOSFOLIPIDIC ÎN 2023 – PARTEA II**
12:20-12:40	**DISCUȚII ȘI CONCLUZII**
12:40-13:00	**ÎNCHIDEREA CONFERINȚEI**