



Erythropoietin and the Role of Inflammation in Anaemia in Patients with Chronic Renal Living in Cote D'ivoire

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Anemia is one of the most common complications of kidney failure. The kidney is responsible for the production of erythropoietin, a key hormone in erythropoiesis. Insufficient production of erythropoietin due to impaired kidney functions and also inflammation could explain this anemia. This study aimed at contributing to a better understanding of the mechanisms of erythropoietin in anemia observed in kidney failure.

Methods: The study population consisted of 138 people: 92 with chronic renal failure (46 not on dialysis, 46 on hemodialysis) and 46 voluntaries as control without kidney failure. Serum concentrations of urea, creatinine, C-reactive protein (CRP), serum iron, ferritin and transferrin were determined using the Cobas C311 Hitachi machine. The erythropoietin assay was performed on the ELISA chain.

Results: Lower mean values of EPO, increased CRP and decreased iron were observed in CKF patients (EPO: 5.66 ± 0.97 mIU / L; CRP: 45 ± 7.46 mg / l ; Iron: 12.46 ± 0.85 μ mol / l), and patients under dialysis (EPO: 9 ± 0.51 mIU / L; CRP: 9 ± 2.66 mg / l; Iron: 10.07 ± 0.54 μ mol / l) compared to controls (EPO: 18 ± 1.29 mIU / L; CRP: 2 ± 0.30 mg / l; Iron: 15.85 ± 0.56 μ mol / l).

Conclusion: Anemia in chronic renal failure is thought to be due to an erythropoietin deficiency but also to an exacerbation of inflammation with a disruption of the iron status.

Keywords: Anemia; chronic kidney failure; Côte d'Ivoire; erythropoietin; inflammation.

1. INTRODUCTION

The increasing incidence and prevalence of chronic renal failure (CKF), the high cost of its treatment and its side effects are major public health concerns around the world. However, it is an unrecognized, insidious disease with few specific symptoms; it is still too often diagnosed at a late stage [1].

Among the complications seen in patients with kidney failure, anemia is one of the most common. It appears in the early stages of the disease and gets worse as the disease progresses to the terminal stage [2].

The production of erythropoietin (EPO) is an important function of the kidney that is very often overlooked. EPO is a signaling molecule that stimulates red blood cells in response to decreased levels of oxygen in the blood. Indeed, it is an anti-apoptotic hormone that promotes the survival, proliferation and differentiation of erythrocyte precursors [3]. Any disruption of this process promotes the onset of anemia characterized by a lower than normal hemoglobin rate. Anemia associated with chronic kidney disease has exposed patients to cardiovascular disease and is associated with poor quality of life, increased hospitalizations, cognitive impairment and mortality [4].

The contribution of recombinant human erythropoietin or erythropoiesis stimulating agents (ESA) in the management of anemia since the end of the 1980s, has thus contributed to reducing the frequency of blood transfusions and their risks, for example increase in hemoglobin level, therefore leading to an improvement in the quality of life of patients with kidney failure [5].

Impaired kidney function appears to influence the level of inflammatory markers, such as serum C-reactive protein (CRP) or interleukin 6 (IL-6) [6]. Anemia in patients with chronic renal failure is a multifactorial process in which it would be important to take into account chronic

inflammation, erythropoietin deficiency, iron metabolism disorders, blood loss during sessions of hemodialysis [4]. Understanding the mechanisms underlying anemia in chronic renal failure is important due to the fact that in some patients' treatment with erythropoiesis-stimulating agents (ESAs) may be at least ineffective or even harmful [7].

In Côte d'Ivoire, there are studies of patients with kidney failure, but very few have focused on measuring erythropoietin (EPO), a key hormone in erythropoiesis. Red blood cell indices provide us with information about their hemoglobin content as well as their level. Abnormal values of these indicate the presence of anemia and give us an idea of the kind of anemia. Ferritin and the degree of transferrin saturation are the best markers of iron content.

The objective of this research is to contribute to a better understanding of the mechanisms of anemia in kidney failure.

2. MATERIALS AND METHODS

2.1 Type and Study Site

This is a descriptive cross-sectional study which was carried out from December 2019 to December 2020 at the department of fundamental and medical biochemistry of the Institute Pasteur of Côte d'Ivoire (IPCI) on 138 people (90 men and 48 women) aged 18 to 74, precisely 46 patients with kidney failure who have never had dialysis (46 CKF patients), 46 chronic hemodialysis patients (46 dialysis patients) and healthy volunteers (46 controls) to serve as control.

2.2 Criteria for the Selection of Subjects

2.2.1 Inclusion criteria

This study included all individuals who met the following inclusion criteria:

Samples from adult hemodialysis patients and those with chronic kidney disease (CKD) as

defined by KDIGO 2017 (GFR < 90 mL/min/1.73m² for more than 03 months). Patients who gave consent for blood sampling. Samples of healthy adults of both sexes with no clinical and biological signs of renal failure who voluntarily agreed to participate in this study.

2.2.2 Non-inclusion criteria

Samples from patients with acute kidney disease, children, pregnant women and kidney transplant patients were not included in the study. People with medical conditions such as diabetes, hypertension, HIV and/or who have not given their consent to participate in the study.

2.3 Blood Samples Collection

The samples were collected from various nephrology units in hospitals in the Abidjan district (Côte d'Ivoire). Venipuncture samples were taken from the bend of the elbow in fasting individuals. In patients with chronic hemodialysis, the sample was taken before and after dialysis, where the frequency was twice weekly. EDTA (Ethylene Diamine Tetra Acetic Acid) tubes were used for complete blood count (CBC) and reticulocyte count on the SYSMEX XN-1000i machine. Dry tubes without anticoagulant were used to measure biochemical parameters (urea, creatinine, reactive C protein (CRP), serum iron, ferritin and transferrin) on the Hitachi Cobas C311 analyser.

2.4 Collecte Blood Including Reticulocytes

Total blood count and reticulocyte count were performed using the XN-1000i automated system from SYSMEX combining the principle of hydrodynamic focusing and fluorescent flow cytometry [8]. According to the WHO classification, anemia occurs when a haemoglobin concentration is less than 12 g/dl in females and less than 13 g/dl in males [9].

2.5 Determination of Biochemical Parameters

Serum concentrations of urea, creatinine, C-reactive protein (CRP), serum iron, ferritin and transferrin were determined using Roche Diagnostic Cobas C311 Hitachi analyzer. The principle is based on the TRINDER reaction which is an enzymatic and colorimetric method with the help of a chromogen. The intensity of colouration or turbidity developed is directly

proportional to the concentration of the measured substance [10].

The total binding capacity of the transferrin and the saturation coefficient of the transferrin were calculated with the following formulae [11]:

$$\text{CTF } (\mu\text{mol / L}) = \text{Transferrin value (g / L)} \times 25.$$

$$\text{CTS (\%)} = (\text{Serum iron / CTF}) \times 100.$$

2.6 Screening for Erythropoietin

Erythropoietin was determined using the Enzyme-Linked ImmunoSorbent Assay (ELISA) method, whose principle is based on the double antibody sandwich technique. The intensity of the color is directly proportional to the amount of conjugate which in turn is directly proportional to the amount of EPO in the sample. A standard curve is drawn by plotting the absorbance of the measured complex in relation to the concentration of the EPO standards. The EPO concentration in the unknown sample is determined by comparing its optical density to the curve of the standard.

2.7 CKD-EPI Equation

The CKD-EPI equation (Chronic Kidney Disease EPIdemiology collaboration) [12] made it possible to estimate the glomerular filtration rate (GFR) and therefore to know the stage of chronic kidney disease in patients with kidney failure who have never undergone dialysis.

2.8 Statistical Analysis

The information was entered using an Excel spreadsheet. The mean values and the mean standard error (mean SEM) for the data were obtained using Graph Pad Prism 8.0 (Microsoft, USA). Statistical analysis of the results was done using the Variance Analysis (ANOVA) followed by the Tukey Multiple Comparison Test. The difference is significant when p-value <0.05.

3. RESULTS

3.1 Epidemiological Data

The mean age for hemodialysis patients was 48±1.99 and for CKD patients was 46±1.86. There were 30 men (65.22%) and 16 women (34.78%) in both hemodialysis patients and CKD patients. The sex ratio M / F was 1.87.

3.2 Stage of the Disease

The stage of chronic kidney disease was calculated in patients with chronic kidney failure, i.e 46 CKF patients not yet on dialysis. It appears that 93.48% and 2.18% were respectively in stages 5 and 4 against 4.34% in stage 3 of chronic kidney disease.

3.3 Haematological Profile

In general, results of hematological markers in patients with chronic renal impairment (CKD and dialysis) show a decrease compared to controls $P < 0.05$ (Table 1). The average hemoglobin (Hb) value was lower for CKD patients than for dialysis patients and controls. Anaemia was present in 94.56% of patients with chronic renal failure (CKD and dialysis patients). Most (48.92%) reported cases had normochromic-normocytic anaemia followed by hypochromic microcytic anaemia (40.21%). However, in a smaller proportion and mostly in dialysis patients, some cases of macrocytic anemia (5.43%) were observed. Erythropoietin concentrations in controls were double that of dialysis patients. The lowest levels were observed in CKD patients (Fig. 1).

3.4 Biochemical Profile

Very high average values of urea and creatin were found in dialysis and CKD patients. For the C-reactive protein (CRP), an increase in its value was observed in dialysis patients over controls (Table 2). This difference is even more pronounced in people with CKD. For iron, there is a significant difference between the values found in controls, dialysis patients and CKD patients. In addition to the fact that the serum iron values are within the range of normal values in the study population, a decrease in its content was nevertheless noted in dialysis patients (53.12%) and CKD patients (37.5%) while no decrease was observed in controls. For ferritin, values in dialysis and CKD patients were significantly elevated relative to controls. The increase in ferritin was 87.75% and 65.62% respectively in CKD patients and dialysis patients compared to 18.75% in controls. Regarding transferrin and the total transferrin binding capacity, the values observed in patients with CKD and dialysis patients were below the normal range, unlike in the controls population. There was no significant difference in the transferrin saturation coefficient (CS) which was within the range of normal values throughout the study population.

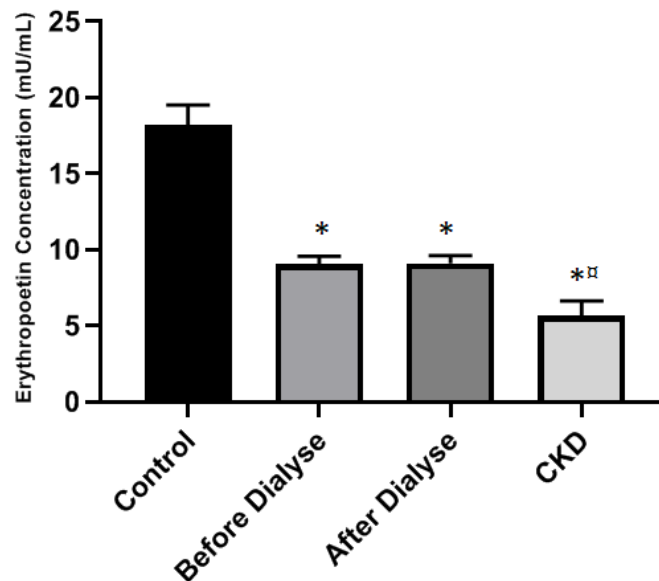


Fig. 1. Erythropoietin concentration of the study population

CKD: patients with kidney disease who have never had dialysis; Dialysis patients: chronic hemodialysis patients;

*: Significant difference between chronic renal failure patients (CKF and dialysis patients) and controls, $P < 0.05$.

α: Significant difference between patients before dialysis and after dialysis and CKF patients $P < 0.05$

Table 1. Average concentrations of hematological markers and erythropoietin in the study population

Parametrs	Control	Before Dialyse	After Dialyse	CKD	p value a	p value b	p value c
Red blood cell (3.8 – 6.0 10 ⁶ /μL)	4,58 ± 0,09	3,46 ± 0,09	3,81 ± 0,11	2,68 ± 0,12	0,0001*	0,0006*	0,0001*
Hematocrit (35 – 54 %)	39 ± 0,88	30 ± 1,02	33 ± 1,21	21 ± 1,31	0,0001*	0,0013*	0,0001
Hemoglobin (12 – 18 g/dL)	13 ± 0,23	9 ± 0,25*	10 ± 0,31*	7 ± 0,33*	0,0001*	0,0001*	0,0001*
MCV (80 – 95 fL)	84 ± 0,67	84 ± 1,08	85 ± 0,85	79 ± 0,96	0,28	0,92	0,0009*
MCHC (32 – 36 g/dL)	33 ± 0,14	31 ± 0,15	31 ± 0,18	32 ± 0,39	0,0001*	0,0081*	0,59
Reticulocytes (25 – 100 10 ⁹ /μL)	61 ± 00	80 ± 0,01	76 ± 00	53 ± 00	0,56	0,72	0,94

CKD: patients with kidney disease who have never had dialysis; Before Dialyse: chronic hemodialysis patients before the dialysis session; After Dialyse: chronic hemodialysis patients after the dialysis session; MCV: mean corpuscular volume, MCHC: mean corpuscular hemoglobin concentration

*: p indicates statistical significance. The difference is significant for $p < 0.05$. a : Control vs chronic hemodialysis patients before the dialysis session, b : Control vs chronic hemodialysis patients after the dialysis session, c : Control vs patients with kidney disease who have never had dialysis.

Table 2. Average concentrations of biochemical markers and EPO in the study population

Parametrs	Control	Before Dialyse	After dialyse	CKD	p value a	p value b	p value c
Creatin (5 - 12 mg/L)	10±0,31	158±4,5	61±3,65	140±9,23	0,0001*	0,0001*	0,0001*
Urea (0,10 - 0,35 g/L)	0,24±0,01	1,57±0,05	0,54±0,04	2,13±0,13	0,0001*	0,02*	0,0001*
CRP (< 6 mg/L)	2±0,30	9±2,66	12±3,44	45±7,46	0,68	0,36	0,0001*
Iron (μmol/l) (M: 11-28; F: 6.6-26)	15,85±0,56	10,07±0,54	10,21±0,61	12,46±0,85	0,0001*	0,0001*	0,0018*
Transferrin (g/L) (M: 2.0-2,7 ; F: 1.9-2.7)	2,40±0,06	1,41±0,07	1,54±0,08	1,26±0,08	0,0001*	0,0001*	0,0001*
CTF (49-69 μmol/L)	61±1,22	35±1,34	37±1,41	32±1,78	0,0001*	0,0001*	0,0001*
CS (20-45 %)	27±1,05	36±5,36	34±4,14	43±3,46	0,33	0,55	0,01*
Ferritin (μg/l) (M: 30-300; F: 20-200)	160±21,34	810 ± 162,5	795±81,44	1215±106,8	0,0001*	0,0002*	0,0001*

CKD: patients with kidney disease who have never had dialysis; Before Dialyse: chronic hemodialysis patients before the dialysis session; After Dialyse: chronic hemodialysis patients after the dialysis session; CTF: Total Transferrin Binding Capacity; CS: Saturation coefficient of transferrin; CRP: C-reactive protein; M: Male; F: Femal

*: p indicates statistical significance. The difference is significant for $p < 0.05$. a : Control vs chronic hemodialysis patients before the dialysis session, b : Control vs chronic hemodialysis patients after the dialysis session, c : Control vs patients with kidney disease who have never had dialysis.

4. DISCUSSION

Almost all of the CKD patients in this study had advanced stage chronic kidney disease were in stages 4 and 5, respectively that is, by that moment kidney replacement therapy is very essential. This could be attributed not only to the low socio-economic level of the populations, but also to the under-medicalization in developing countries with the lack of awareness of the population in the face of the warning symptoms of kidney disease [13].

The anemia was of the normochromic normocytic type in the majority of the cases followed by a hypochromic microcytic anemia and few cases in a macrocytic anemia mainly in dialysis patients. These results agree with those of Shastry and Belurkar [14] who also found the presence of these three types of anemia in their study population. Anemia in CKD is usually normochromic, normocytic, but sometimes it can be microcytic hypochromic due to lack of iron associated with insufficient production of red blood cells caused by reduced EPO activity in the bone marrow [14]. Patients with kidney failure may also present macrocytic anemia due to vitamin B12 or folic acid deficiency. This deficiency is uncommon, occurring in less than 10% of dialysis patients. Folic acid deficiency may occur primarily in dialysis patients, since serum folate levels are also reduced after dialysis [15].

In this study, anemia was regenerative. The reticulocytes count is useful for estimating the functional integrity of the bone marrow. A decrease in reticulocytes in blood level reflects a bone marrow defect in anemic patients [16].

The levels of red blood cells, hematocrit and hemoglobin observed were significantly higher in controls than in chronic renal failure (CKD and dialysis patients), this is corroborated to the work of Behera [17]. The main cause of low red blood cell count in chronic kidney disease is impaired erythropoietin synthesis and reduced red blood cell survival. The hormone erythropoietin, the main humoral regulator of red blood cell production, helps maintain red blood cell viability by delaying DNA cleavage that normally occurs in the CFU-E (colony forming unit-erythroid) stage of erythropoiesis [18]. In the absence of EPO, DNA cleavage is rapid and results in cell death.

The decrease in serum erythropoietin (EPO) concentrations observed in CKD patients is considered to be the main cause of the development of kidney anemia. Erythropoietin (EPO) is a hormone produced by specialized type I interstitial fibroblasts in the cortex and the outer layer of the renal medulla. As kidney disease progresses, kidney mass decreases, which reduces the production of EPO and creates its deficit [3]. However, in our study, the erythropoietin concentration in dialysis patients was higher than that of CKD patients, this would be due to the use of recombinant erythropoietin in hemodialysis centers. This is because erythropoietin (EPO) is a critical factor that regulates the number of red blood cells associated with its integration into the oxygen-carrying system. The main stimulus for high EPO synthesis is tissue hypoxia, which normally leads to an exponential increase in serum EPO levels [19]. This feedback is affected in patients with pathological conditions involving the kidneys, and the developing anemia is not properly compensated by a sufficient increase in EPO production.

The increase in CRP, ferritin and on the other hand, the decrease in serum iron concentrations, transferrin, total iron binding capacity to transferrin (CTF), observed in the majority of patients with chronic renal failure in this study, are characteristics of an inflammatory profile. In the early hours of the occurrence of inflammation cytokines, including tumor necrosis factor α (TNF- α), interleukin-1, interleukin-6 and interferon- γ , are produced by inflammatory cells [20]. This can be explained firstly, by systemic immune activation which leads to profound changes in iron trafficking, resulting in iron retention in macrophages and reduced intestinal absorption of dietary iron. The work of Mayeur et al in 2014 showed that interleukin-6 induced the expression of hepcidin by a signal transducer and a transcription activator 3 (STAT3) [21]. Hepcidin exerts its iron regulating effects by binding ferroportin, an iron transporter to the cell surface, causing cellular internalization and degradation of ferroportin. Thus, increasing hepcidin concentrations, inhibits iron absorption in the duodenum where ferroportin is needed to deliver the dietary iron absorbed into the circulation, and they also act on macrophages to block the release of recycled iron from senescent erythrocytes in plasma [22]. This set of mechanisms promotes the decrease in serum iron concentration and the ability to bind iron to transferrin [23].

5. CONCLUSION

Anemia, a common complication in chronic kidney disease, is primarily due to decreased erythropoietin levels. However, inflammation plays an important role in the development of anemia. Determining erythropoietin levels and inflammatory parameters could improve the medical management of chronic kidney disease in Côte d'Ivoire.

ETHICS APPROVAL AND CONSENT

The study was authorized for implementation by the national ethics committee for life and health sciences (CNESVS) assigned number 023-20 / MSHP / CNESVS-km. Informed Consent has been obtained from the individuals for the use of blood samples for the research.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Sumaili EK, Krzesinski JM, Cohen EP & Nseka NM, épidémiologie de la maladie rénale chronique en République démocratique du Congo : une revue synthétique des études de Kinshasa, la capitale. *Néphrologie & Thérapeutique*. 2010;6:232.
2. Janus N, Launay VV. Complication de l'insuffisance rénale chronique : l'anémie et ses traitements. *Journal de Pharmacie Clinique*. 2011;30:229.
3. Atkinson MA, Warady BA. Anemia in chronic kidney disease. *Pediatric Nephrology*. 2018;33:227.
4. Babitt JL, Lin HY, mechanisms of anemia in CKD. *JASN*. 2012;23:1631.
5. López-Gómez JM, Abad S, Vega A. New expectations in the treatment of anemia in chronic kidney disease. *Nefrologia*. 2016;36:232.
6. Panichi V, Migliori M, De Pietro S, Taccola D, Bianchi AM, Norpoth M, Metelli MR, Giovannini L, Tetta C, Palla R. C-reactive protein in patients with chronic renal diseases. *Ren. Fail.* 2001;23: 551.
7. Caldararu CD, Tarta DI, Gliga ML, Tarta C, Carasca E, Albu S, Hutanu A, Dogaru M, Dogaru G. Comparative analysis of hepcidin-25 and inflammatory markers in patients with chronic kidney disease with and without anemia. *Acta Med. Marisiensis*. 2017;63:10.
8. Ormerod MG, Sun XM, Snowden RT, Davies R, Fearnhead H, Cohen GM. Increased membrane permeability of apoptotic thymocytes: A flow cytometric study. *Cytometry*. 1993;14: 595.
9. WHO, UNICEF & UNU, Iron deficiency anaemia. Assessment, prevention and control. A guide for Programme Managers. 2001;132.
10. Deyhimi F, Arabieh M, Parvin L. Optimization of the Emerson Trinder enzymatic reaction by response surface methodology. *Biocatal. Biotransfor.* 2006;24:263.
11. Wagner A. le rôle du laboratoire dans l'exploration du métabolisme du fer. *Revue de l'ACOMEN*. 2000;6:23.
12. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, Coresh J. CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration), a new equation to estimate glomerular filtration rate. *Ann Intern Med*. 2009;150:604.
13. Ackoundou-N'Guessan KC, N'Zoue S, Lagou AD, Tia, WM, Guei MC, Coulibaly PA, Gnonsiahe DA. Épidémiologie de l'hypertension artérielle non contrôlée au cours des maladies rénales chroniques chez des patients admis dans une unité de néphrologie d'Afrique noire : une étude rétrospective de 479 patients. *Néphrol ther.* 2014;745:1.
14. Shastry I, Belurkar S. The spectrum of red blood cell parameters in chronic kidney disease: A study of 300 Cases. *J Appl Hematol*. 2019;10:61.
15. Zadrazil J, Horak P. Pathophysiology of anemia in chronic kidney diseases: A review. *Biomed Pap Med*. 2015;159:197.

16. Means RT. Anemia of renal failure/chronic kidney disease. In R.T. Means Jr., éd. Anemia in the Young and Old: Diagnosis and Management. 2019;147.
17. Behera PB. Hematological profile in patients of chronic kidney disease with its severity in a tertiary care hospital, north odisha. Asian J Pharm Clin Res. 2020;13:182.
18. Lombardero M, Kovacs K, Scheithauer BW. Erythropoietin: a hormone with multiple functions. Pathobiology. 2011;78:41.
19. Jelkmann W. Regulation of erythropoietin production. J Physiol. 2011;589:1251.
20. Tomas G, Anemia of Inflammation. N Engl J Med. 2019;381:1148.
21. Mayeur C, Lohmeyer LK, Leyton P, Kao SM, Pappas AE, Kolodziej AS, Spagnolli E, Yu B, Galdos RL, Yu PB, Peterson RT, Bloch DB, Bloch KD, Steinbicker AU. The type I BMP receptor Alk3 is required for the induction of hepatic hepcidin gene expression by interleukin-6. Blood. 2014; 123:2261.
22. Ueda N, Takasawa K. Role of hepcidin-25 in chronic kidney disease: Anemia and beyond. Curr. Med. Chem. 2017;24: 1417.
23. Marioa N. Marqueurs biologiques pour le diagnostic des troubles du métabolisme du fer. Revue Francophone des Laboratoires. 2012;442:39.

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