TITLE PAGE

Title: Plasma creatinine below limit of quantification in a patient with acute kidney injury

Running title: Underestimated plasma creatinine

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1. CASE DESCRIPTION

We present a case of an 80-year-old man, suffering from chronic kidney disease (CKD) (estimated Glomerular Filtration Rate, eGFR: 40 mL/min/1.73 m^2) and hypertension without nephrological follow-up. He had chronic diarrheas for few years without definitive diagnosis of inflammatory bowel disease (IBD). As he had increased C-reactive protein (CRP), he was however treated with oral Pentasa® (2 g twice a day) for ten months. The anti-inflammatory agent Pentasa® (mesalazine or 5-aminosalicylic acid, 5-ASA) is a reference treatment to obtain and maintain remissions of ulcerative colitis and Crohn's disease [1]. The patient was also treated with an angiotensin converting enzyme inhibitor, a beta-blocker and a loop diuretic. Upon of his general practitioner's recommendation, he was admitted to the emergency room of Bordeaux University Hospital for an acute kidney injury (AKI) evidenced by plasma creatinine at 9.36 mg/dL (827 µmol/L) in his local laboratory, 5 days before the hospitalization. On arrival, the patient was confused and showed signs of dehydration (skin turgor, dry tongue) and liquid stools. The patient was afebrile, his blood pressure was 114/51 mmHg, the heart rate was 68 beats per minute and he presented an oliguria (urine output less than 500 mL per day).

Surprisingly, he had non-measurable plasma creatinine by the enzymatic method (result below limit of quantification, LOQ, Table 1) but the Jaffe's method successfully determined creatinine concentration at 9.90 mg/dL (875 µmol/L), in agreement with the previous determination. In the same way, plasma urea was 88.77 mg/dL (31.7 mmol/L). Hyperkaliemia (7.7 mmol/L) impacting the electrocardiogram (peaked T-waves) and a severe metabolic acidosis (pH=7.09, alkaline reserve 8 mmol/L) with an increased anion gap at 25 mEq/L (25 mmol/L) were also observed. The biochemical follow-up of the patient from day 1 to day 5 of hospitalization

showed that creatinine measurement by the enzymatic method was restored and similar to the Jaffe's method from the 4th day of hospitalization (Table 1). In addition to the medical treatment (intravenous calcium gluconate, insulin/glucose, salbutamol, oral chelating agents) and rehydration to correct a potential pre-renal acute renal injury, the patient needed an extrarenal epuration to normalize metabolic disorders and water retention for nine days (5 sessions of intermittent hemodialysis from day 5 to day 14). Investigation of renal impairment and its causes confirmed that the patient did not take any drugs known to be associated with kidney injury such as alcohol poisoning, others anti-inflammatory drugs, or antibiotics. An abdominal computed tomography scan eliminated post-renal AKI. There was no evidence of proteinuria and autoimmune disease. Moreover, hepatitis B and C serologies were negative, and serum complement levels were within the normal limits. Renal function gradually improved with plasma creatinine dropping to 2.25 mg/dL (199 µmol/L) (eGFR 27mL/min/1.73m^2) when the patient was discharged from the hospital.

2. DISCUSSION

2.1. Renal failure

The AKI observed in our patient could originate from his pathological status and/or have iatrogenic origin.

Renal failure is an infrequent complication of IBD. It is often related to ureteral obstruction by calcium oxalate stones or fibrous adhesions [2]. It may also be linked to interstitial nephritis secondary to anti-inflammatory drugs, such as Pentasa® (5-ASA) [1], with an incidence less than 0.5% per patient and per year [3]. Pentasa® nephrotoxicity by interstitial nephritis mostly occurs within the first 12 months after treatment setup, but can also appear after a few years [4]. In the latter case, the

duration of treatment proves pejorative in the recovery of renal function after cessation of treatment [4]. Therefore, it is recommended to monitor renal function twice a year (creatinine dosage and 24-hour proteinuria test) and to stop treatment in the event of abnormalities [5]. In addition, the possibility of nephrotoxicity and its potential severity justifies the assessment of renal function before starting any treatment with 5-ASA. In this way, all Mesalazine-based treatments are not recommended by the French national health comity (HAS, Haute Autorité de Santé) for patients with severe renal failure [6]. Noticeably, although the case reported here regards a patient with CKD, he did not undergo regular renal function exploration. Thus, it is possible that the reported AKI is the combined result of IBD complication and 5-ASA treatment side effects.

2.2. Pentasa® impact on creatinine measurement

As stated above, renal function follow-up is crucial for patients under 5-ASA. Accurate and precise creatinine measurement is a routine parameter of kidney function evaluation. Two types of standardized creatinine methods are currently available: colorimetric Jaffe or enzymatic creatinine assays. On the first day of hospitalization, the creatinine result of our case, obtained by an enzymatic assay, was very surprising (< 0.1 mg/dL, *i.e.* < 9 µmol/L, Table 1), considering the clinical context and the anterior result in a local laboratory. The Jaffe assay found results compatible to the clinical status at 9.90 mg/dL (875 µmol/L, Table 1).

Considering the fact that the strong negative interference observed on creatinine measurement gradually disappeared, that the Jaffe test was unaffected and that the enzymatic assay was normalized concomitant to the discontinuation of

5-ASA administration (from day 3, Table 1), we hypothesized that 5-ASA could interfere with creatinine enzymatic assays.

We realized interference experiments by adding increasing doses of pure 5-ASA to plasma from patients naïve of 5-ASA treatment (Figure 1). As hypothesized, creatinine concentrations by the enzymatic method decreased with increasing doses of 5-ASA. As expected, no interference was found with the Jaffe's method (Figure 1A).

One of the known mechanisms of action of 5-ASA is its antioxidant properties carried by its ability to scavenge a variety of free radicals [7]. It reacts with various oxygen-derived species, such as hydrogen peroxide (H_2O_2), superoxide radicals, hydroxyl radicals, hypochlorous acid and peroxyl radicals, which are some of the mediators produced in the inflamed colon mucosa of IBD patients [8].

Enzymatic assays combine serial reactions aimed at converting creatinine into H_2O_2. Three enzymes are successively involved: creatininase, creatinase and sarcosine oxidase. The last reaction, catalyzed by a peroxidase, also called the Trinder reaction, uses H_2O_2 to convert an uncolored dye in a colored compound proportionally to creatinine concentration. In the Jaffe method creatinine which forms a yellow-orange complex with picric acid in alkaline conditions, does not use the Trinder reaction. The intensity of the color is simply proportional to creatinine concentration. Of note, the Trinder reaction is shared by other multi-enzymatic biochemical tests (Table 1). Thus, creatinine and other enzymatic assays rely on H_2O_2 being the exclusive substrate of the peroxidase [9].

We believe that, in the reported case, AKI resulted in 5-ASA plasma accumulation by elimination impairment. Recommended doses of 5-ASA are 4g per

day for induction and 2g per day for maintenance treatment of Crohn disease. Our patient had been receiving 4 g per day for 10 months at the time of hospitalization, regardless of his chronic renal disease. After a single administration of 4g of 5-ASA, plasma concentrations were estimated to reach about 5 µg/mL in elderly patients (75 years and older, with normal kidney function), 12.5 hours after oral administration [10]. We can suppose that 5-ASA elimination follows creatinine and circulating 5-ASA in our patient may be up to 10 folds higher than a person with normal kidney function. Interferogram experiments with 5-ASA spiking to reach up to 100 µg/mL confirmed the negative interference of 5-ASA from 10 µg/mL (Figure 1A).

In turns, high concentrations of plasma 5-ASA scavenged the intermediate H_2O_2 produced during the enzymatic creatinine test. This hypothesis is reinforced by the fact that no interference was detected with the colorimetric Jaffe's method in all the tested samples (Table 1) and in the interferogram analysis (Figure 1A). The interference was majored in plasma containing high creatinine levels, suggesting that the interference is proportional to H_20_2 production (Figure 1A). This hypothesis is also in agreement with the strong discordance between results of Trinder reaction-based measurements (uric acid, lactate, lipase, triglycerides, total and HDL cholesterol) at day 1 and day 5 (Table 1). Interference experiments confirmed the negative effect of 5-ASA on these Trinder reaction-dependent assays, with strong impact on triglycerides, lipase and total cholesterol (Figure 1B).

In the same way, parameters measured with Trinder-independent reactions, in particular urea (UV spectrophotometry) and chloride anion (potentiometry) showed little variations within the same period. The variations observed for natremia and total protein are attributable to rehydration of the patient, whereas kalemia and alkaline reserve normalization were due to acidosis correction (Table 1). Also of note, similar

negative interferences were previously proposed with drugs such as calcium dobesilate, ethamsylate, aspirin or catecholamines. More generally, p-diphenols-containing drugs seem to interfere with diagnostic tests utilizing the Trinder reaction [11] [12] [13] [14] [15]. As 5-ASA is a salicylate derivative with close structural homology with these molecules, it is possible that according to the general status of the patient, additional negative interference contributed to interfere with Trinder reaction-based assays. This hypothesis is supported by the fact that patients with AKI display 2- to 12-fold increases in catecholamines and their metabolites [16].

The patient had acidosis with an increased anion gap, which happens when unmeasured anions are present in the plasma, including acids. It is unlikely that it was a lactate-related acidosis since lactatemia was in normal range, although probably underestimated (5-ASA interference, Table 1). Interestingly, 5-ASA stands for 5-amino-2-hydroxybenzoic acid with pKa_1=2.02 and pKa_2=5.87. Its physiological charge is negative, which is in favor of increasing the anion gap.

3. CONCLUSION

The results reported here highlight the strong necessity to know the exact drugs taken by patients in case of AKI complicated by non-lactic acidosis. If salicylate derivatives are part of the patient's prescriptions, in addition of having deleterious effects on the acid-base status of the patient, they may interfere with biochemical parameters which measurements depend on the Trinder reaction. Their potential underestimation should be considered in the biological-clinical patient management.

This study highlights the important fact that parameters, which measurement depends on the Trinder reaction, should be interpreted with the knowledge of patient's treatment, in particular salicylate derivatives.

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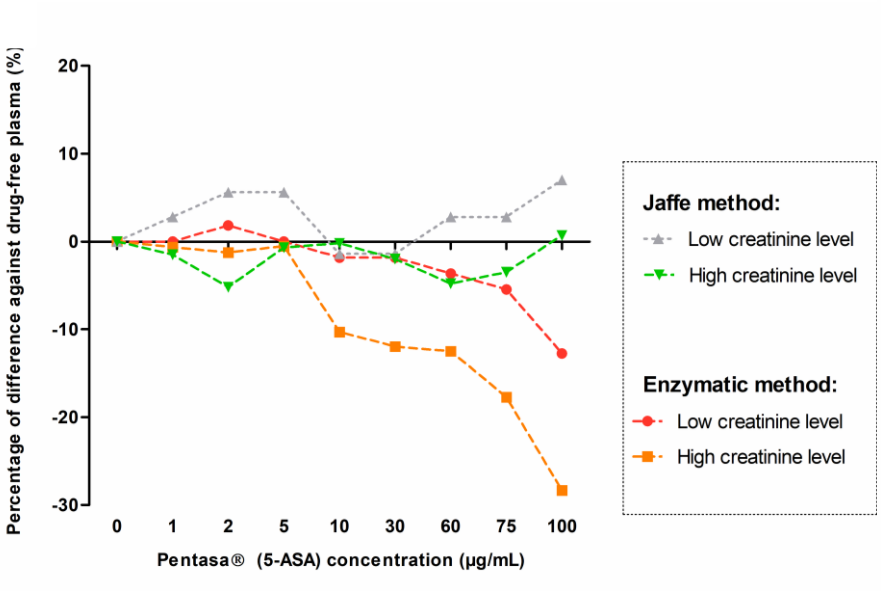
DECLARATION OF INTEREST

All the authors declare no conflict of interest.

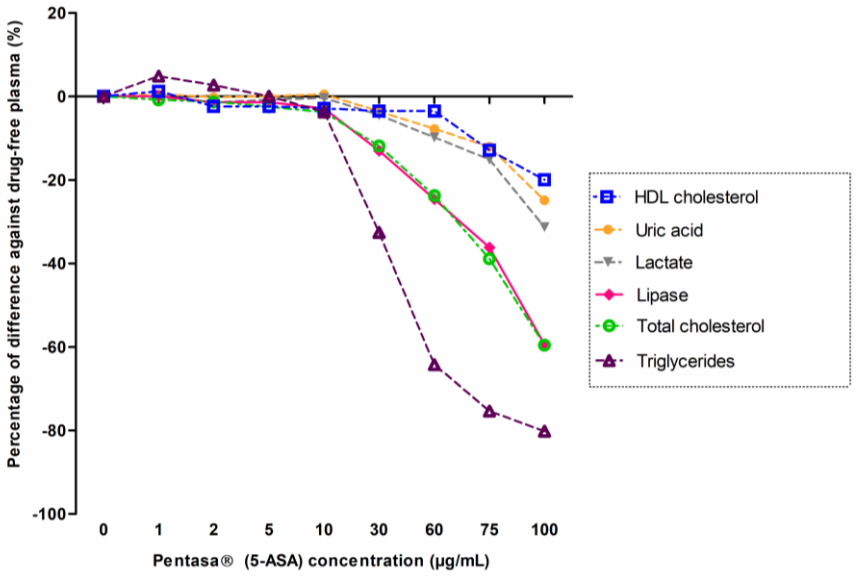
FIGURE CAPTION

Figure 1. Effect of exogenous addition of Pentasa® (5-ASA) in drug-free plasma on Trinder reaction-dependent tests. (A) Quantification of low (0.63 mg/dL) and high (6.8 mg/dL) creatinine levels by enzymatic method compared to colorimetric Jaffe method. (B) Quantification of uric acid, lactate, lipase, triglycerides, total and HDL-cholesterol levels. All the tests were realized on Abbott Architect system, except for Jaffe method which was analyzed on Randox Monaco system.

A


Percentage of difference against drug-free plasma (%)
20
10
0
-10
-20
-30
0
1
2
5
10
30
60
75
100
Pentasa® (5-ASA) concentration (µg/mL)
Jaffe method:
Low creatinine level
High creatinine level
Enzymatic method:
Low creatinine level
High creatinine level

B


Percentage of difference against drug-free plasma (%)
20
0
-20
-40
-60
-80
-100
0
1
2
5
10
30
60
75
100
Pentasa® (5-ASA) concentration (µg/mL)
HDL cholesterol
Uric acid
Lactate
Lipase
Total cholesterol
Triglycerides

C

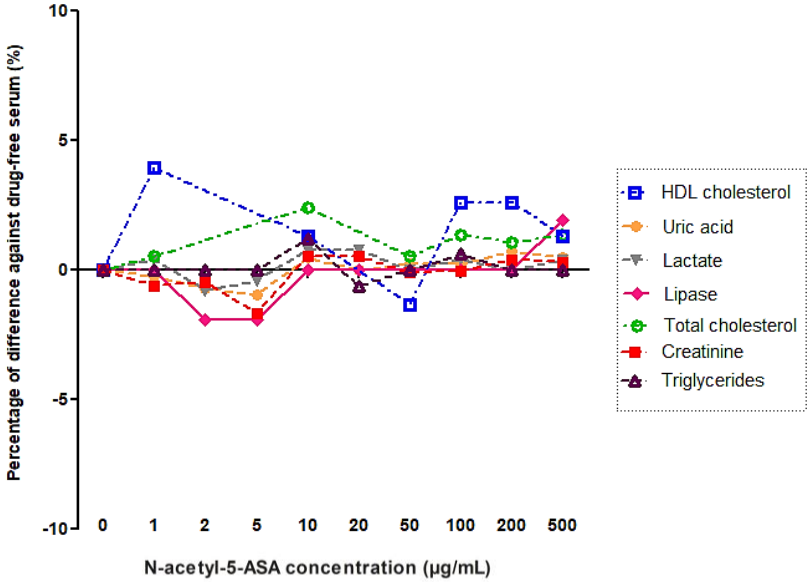

Percentage of difference against drug-free serum (%)
10
5
0
-5
-10
0
1
2
5
10
20
50
100
200
500
N-acetyl-5-ASA concentration (µg/mL)
HDL cholesterol
Uric acid
Lactate
Lipase
Total cholesterol
Creatinine
Triglycerides

Table 1. Evolution of biochemical parameters in patient plasma during hospitalization

	BIOCHEMICAL PARAMETER	TRADITIONAL MASS UNIT (SI UNIT)	ANALYTICAL METHOD	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	% OF DIFFERENCE BETWEEN DAY 5 AND DAY 1
			5-ASA treatment	Yes	Yes	No	No	No Pre-dialysis	
Parameters measured for the patient follow-up	**Creatinine**	mg/dL (µmol/L)	Enzymatic Trinder reaction	< 0.10 (< 9)	< 0.10 (< 9)	6.10 (539)	10.99 (972)	11.61 (1026)	>99.1% *
		mg/dL (µmol/L)	Colorimetry Jaffe's method	9.90 (875)	-	11.28 (997)	10.97 (970)	-	
	Urea	mg/dL (mmol/L)	Enzymatic UV	88.77 (31.7)	89.05 (31.8)	88.21 (31.5)	94.65 (33.8)	93.81 (33.5)	5.3%
	Sodium	mmol/L	Potentiometry	141	143	144	148	147	4.1%
	Potassium	mmol/L	Potentiometry	7.77	6.85	5.98	4.96	3.95	96.7%
	Chloride	mmol/L	Potentiometry	116	119	117	118	117	0.9%
	Alkaline reserve	mmol/L	Enzymatic UV	8	8	8	10	12	33.3%
	Anion gap	mEq/L (or mmol/L)	Calculated	25	23	25	25	22	12%
	Total protein	g/dL (g/L)	Colorimetry	8.1 (81)	7 (70)	7.5 (75)	7.1 (71)	6.6 (66)	22.7%
	C-Reactive Protein	mg/L	Immunoturbidi-metry	88	-	143	-	101	12.9%
Parameters measured for interference test	**Uric acid**	mg/dL (µmol/L)	Enzymatic Trinder reaction	< 1.01 (< 60)	-	-	-	7.45 (443)	> 86.4% *
	Lactate	mg/dL (mmol/L)	Enzymatic Trinder reaction	7.48 (0.83)	-	-	-	19.01 (2.11)	60.6%
	Lipase	U/L	Enzymatic Trinder reaction	< 4	-	-	-	66	>93.9% *
	Triglycerides	mg/dL (mmol/L)	Enzymatic Trinder reaction	53.1 (0.6)	-	-	-	125.7 (1.42)	57.7%
	Total cholesterol	mg/dL (mmol/L)	Enzymatic Trinder reaction	59.54 (1.54)	-	-	-	101.7 (2.63)	41.4%
	HDL cholesterol	mg/dL (mmol/L)	Enzymatic Trinder reaction	13.53 (0.35)	-	-	-	20.11 (0.52)	32.7%

The concentrations were determined using Architect systems (Abbott diagnostics, Rungis, France) on plasmas collected on Barricor tubes (Becton Dickinson, Le-Pont-de-Claix, France). The determination of creatinine by Jaffe's method was realized using the Randox alkaline picrate method (Randox, Roissy-en-France, France). * Values calculated correspond to the minimal % of difference (result below LOQ at day 1).