CASE STUDY

Cephalosporin interference in the Jaffe creatinine method: a case study

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ABSTRACT

This is a case of apparent acute kidney injury (AKI) due to interference with the Jaffe creatinine method. In spite of improvements in the Jaffe assay it is prone to interferences that may lead to erroneous results. Inaccurate creatinine results lead to incorrect estimation of the glomerular filtration rate (eGFR) which is the hallmark of assessing kidney function and detecting AKI. Therefore, scientists need to be aware of the circumstances that can jeopardise analytical analysis particularly when it comes to specific filtration markers, such as creatinine.
Key words: eGFR, kidney failure, interference, creatinine, analytical variables, Jaffe assay.

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INTRODUCTION

Creatinine is a waste product of muscle that is normally filtered from the blood by the kidneys. Urea is a waste product of protein metabolism that is also removed by the kidneys. Both increase when kidney function is compromised although urea is less specific to kidney dysfunction because it can also increase under other conditions e.g. gastrointestinal bleeding (1). Nevertheless blood creatinine concentration along with blood urea concentration have historically been used as first-line tests to diagnose and monitor kidney disease (2).

When kidney function is compromised, creatinine is not filtered leading to high blood creatinine levels. However, blood creatinine alone does not accurately reflect kidney function in some clinical situations as it is influenced by several pre-analytical factors associated with muscle mass such as age e.g. it can be low in the frail elderly in spite of impaired kidney function, sex e.g. it is lower in females compared to males, race e.g. it is higher in Africans because of a physiologically higher muscle mass compared to Caucasians, and body size e.g. it is very low in children and neonates because of their small muscle bulk, and haemodynamics e.g. it is lower in pregnancy than the non-pregnant state because of increased filtration (3). Thus, creatinine has high specificity but is not a sensitive marker for kidney function.

Elevated creatinine in muscular individuals can incorrectly suggest kidney injury while the low or normal creatinine concentration in thin individuals can mistakenly suggest healthy kidney function (4). For instance, patients with a relatively lower muscle mass can have a serum creatinine concentration within normal reference range, although their renal function is severely compromised (5). Furthermore, an increased serum creatinine is suggestive of kidney damage but only occurs after a significant proportion of renal function has already been lost i.e. there is a lag phase in which creatinine levels are “normal” but the kidneys are deteriorating (5).

The rate of glomerular filtration is a marker for how effectively the filtering units, nephrons, filter (remove) unwanted material over a period of time (1). The relationship between serum creatinine is inversely proportional to eGFR, which starts to drop before creatinine significantly rises in the early stages of injury, proving eGFR calculations to be more sensitive than creatinine alone (4).

This is because eGFR includes other factors apart from creatinine that influence kidney function namely age, sex. One of the most important tools for measuring renal function is the eGFR. eGFR helps screen for and diagnose kidney injury and monitor the progression of chronic kidney disease (CKD) (5). CKD is defined as an eGFR less than 60 mL/min/1.73m^2 for over three months (6).

Several equations are now widely used to indirectly measure eGFR using serum creatinine concentration. The historical timeline starts from the Cockcroft-Gault (CG) equation followed by the Modification of Diet in Renal Disease (MDRD), then the *modified* MDRD (with a factor for creatinine measurement) equation when creatinine measurement became standardised, and finally the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations (7). CG equation is not often used in routine clinical practice because it requires a patient’s weight, which is not usually provided in request forms and can be a labile (5). The main reason for the continued need for the CG equation is that most recommendation for drug dosing use it hence pharmacists still rely on it heavily (6).

The MDRD equation provides an eGFR based on serum creatinine concentration, age, sex and is normalised adult body size of 1.73m² (3). Ideally it includes the patient’s ethnicity but this is another parameter that is not usually provided on request forms and hence the equation in use does not factor it in (6). Because MDRD does not require a measurement of body weight or height, laboratories can report eGFR using information that is routinely provided. A limitation of the MDRD (and its modified version) was that it under estimated kidney function in healthy individuals (5). The reason for that was that the population used to generate the equation was mostly unwell. The CKD-EPI equation was found to be more accurate than the MDRD equation for patients with an eGFR of more than 60 mL/min/1.73m^2 i.e. for healthy people, and for females and younger people (6). CKD-EPI also factors in creatinine, age and sex. Nonetheless, improvements and further modification of the formulas need to be researched for better refining eGFR (8). Consequently, according to Australasian eGFR Consensus reporting eGFR using CKD-EPI equation is recommended and

became standard in most laboratories in Australasia, all laboratories in New Zealand use the CKD-EPI equation (7).

eGFR is done through computer-based calculations. When the analysers connect to laboratory information system (LIS), the test results of serum creatinine can automatically generate eGFR (5). The automation of eGFR enables a high workload and effective reporting of the results for better patient management. This also reduces potential errors of manually calculating the eGFR (8).

Even though eGFR has proven to be one of the best single measures for kidney function it has its limitations that are common to all equations. These limitations include all pre-analytical, analytical and post-analytical challenges that creatinine measurement introduces; their lack of suitability for use in pregnant women, children and in the unstable patient; and their limited application to some ethnicities. It is prudent to interpret eGFR against the backdrop of these limitations (6).

CASE STUDY

The purpose of this case study was to investigate a sudden drop in eGFR with a high creatinine concentration of 873 umol/L.

Mr X was a 70-year-old male patient with hypertension, CKD, longstanding emphysema, chronic sinusitis, gastroesophageal reflux disease, and diverticulitis. He had no history of recent surgery. He did not have diabetes mellitus. He was a lifelong smoker who quit smoking in 2004. A computed tomography scan in 2014 showed severe emphysema with mild sub-plural fibrosis. The emphysema was treated with salbutamol sulphate, fluticasone and tiotropium bromide; drug inhalation medication for chronic obstructive pulmonary diseases (9). His longstanding hypertension and emphysema probably contributed to his CKD (5,10). He was prescribed the antihypertensive medication, doxazosin mesylate (11). Mr X's eGFR had been in the 60-78 ml/min/1.73m^2 range for at least the previous 8 years.

Laboratory findings

On 4th December 2011, upon routine monitoring, Mr X was found to have an eGFR of 5 mL/min/1.73m^2, an approximate 93% reduction from his usual eGFR. This drop was immediately communicated to his General Practitioner who admitted him to the hospital the same day for assessment. Table 1 summarises Mr X's results. A repeat renal profile demonstrated a return of the eGFR to the patient's baseline. The patient was discharged within hours.

Table 1. Timeline of renal function tests

	RR	Lab A	Lab A	Lab B	Lab A	Lab B	Lab A	Lab A
Date		**25/5/10**	**4/2/11**	**21/9/11**	**4/12/11**	**4/12/11**	**26/9/12**	**21/3/13**
Sodium mmol/L	135-145	138		143	**164**	144	140	140
Potassium mmol/L	3.5-5.2	4.8		4.3	3.9	4.2	4.8	4.5
Creatinine umol/L	60-105	102	86	89	**873**	87	99	87
eGFR mL/min/ 1.73m^2	> 90	**64**	**78**		**5**		**66**	**76**

RR= reference range.

The Jaffe assay, a colorimetric assay used for measuring creatinine, is prone to interferences due to glucose, ketoacids, albumin, and by antibiotics like cefoxitin (cephalosporin) and streptomycin, and ascorbic acid, that provide an inaccurate figure for creatinine (1). All these possible interferences can potentially interfere with alkaline picrate to give false increase in serum creatinine (4). Different companies have attempted to modify the reaction to minimise interferences but they have never been totally eliminated. However, creatinine remains the most convenient marker at the present time and evidence has demonstrated that it can be used to calculate eGFR (5). Enzymatic assays may be used instead of the Jaffe method to avoid the effect of most interferences such as bilirubin but they are less available and usually more expensive than Jaffe technology (3).

DISCUSSION

As demonstrated in Table 1, on the same day there were different test results obtained from two different laboratories. Inter-laboratory differences, in addition to pre-analytical, analytical and post-analytical errors, should be considered for Mr X's discrepant results. Possibilities of pre-analytical errors include sample mix up or contamination from a urine sample leaking if placed in the same bag as the blood sample. Consuming a high protein meal before a blood test is a patient related factor which may have played an important role in the increase in serum creatinine and drop in eGFR in Mr X's case.

The analytical phase may also be prone to inaccuracy due to interference in the assay as stated above. Furthermore, the technician or scientist needs to monitor and check the analyser for the results for calibration and quality control abnormalities as inaccuracies in creatinine measurement reflect on the eGFR calculation.

Post-analytical phase may also be considered to see if there were any information technology (IT) errors as transcription errors are unlikely to occur in fully automated laboratories.

Hypertensive kidney damage happens over time and so cannot explain the sudden drop in eGFR. Hypertension contributes significantly to kidney failure by scarring and narrowing of the blood vessels in the kidneys (1). If the kidneys blood pressure is impaired, the normal homeostasis of the kidney is at risk causing glomerular diseases (12).

AKI seemed the most likely explanation assuming the sudden drop in eGFR was genuine and not due to an analytical error. AKI is defined as a drop in eGFR within a short time frame and is potentially reversible condition and timely intervention is warranted (13).

After the discrepancy in creatinine measurement between the laboratories was discovered and the investigation for apparent interferences or errors did not reveal any, the test requestor was contacted who subsequently discussed the circumstances with the patient. The patient reported recently seeing another doctor.

The flawed diagnosis was due to interference of cephalosporin with the Jaffe creatinine method at Lab A with abnormally high creatinine level and low eGFR due to cephalosporin falsely increasing creatinine measurements (4). The test requestor was unaware of the antibiotic prescription and unaware of the effect cephalosporins can have on the Jaffe method.

It was noted that cephalosporin was given intravenously a couple of hours before his blood test. The antibiotic was prescribed by a locum doctor and was not communicated to the patients' General Practitioner who had requested the renal function test months in advance as part of routine monitoring. A few more hours had passed by the time the patient was re-tested in hospital, reducing the potential effect of the antibiotic on the laboratory Jaffe method. Furthermore, different platforms using the same basic method have different assay modifications and it is possible that in this case the hospital laboratory platform demonstrated less interference with cephalosporins.

Limitation

While clinical notes did not suggest the presence of interfering substances, such as high glucose, ketones, or ascorbic acid, it would have been ideal to test for them. However, by the time the investigation was underway the sample was aging and its volume was insufficient for such testing.

This case report presents findings for one patient but there are probably other similar scenarios in our everyday practice that go undetected. Different platforms do not have identical clinical results for the same analyte. This is because laboratories are different in terms of the methods used on different machines, differences in modifications (often patented technology) for the same method on different machines, in addition to differences in processes and operators; all these factors result in slightly different results (14). In case of known interfering agents, like antibiotics with the Jaffe method, it may be worth considering to what degree the blood antibiotic level, in this case a cephalosporin, interferes in either platform and quantitate the change in concentration with time elapsed after a specified dose. This may help clinicians choose a suitable platform if their patients are on cephalosporins (or streptomycin).

Cystatin C has been proposed as a potential alternative for serum creatinine as a filtration marker. Most studies show that serum levels of cystatin C are not affected by muscle mass and more closely correlated with GFR than serum creatinine. However, comparison studies between two markers are still ongoing to ensure its accuracy (15).

CONCLUSION

This case study highlights the significance of assay interferences that affect creatinine measurement using the Jaffe method that reflect on the eGFR calculation; an important limitation of eGFR. It is important to be aware of pre-analytical and analytical errors, and interferences that can jeopardise analysis when it comes to specific filtration markers, such as creatinine. Further studies need to be carried out to investigate what blood level with varying amounts of cephalosporin can affect the Jaffe assay.

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