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Clinical thresholds for pseudohyperkalemia and pseudonormokalemia in patients with thrombocytosis

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ABSTRACT

Background

The lysis of platelets during in vitro coagulation leads to increased potassium concentrations.

We aimed to establish the cut-off value for platelet count interfering serum potassium and to estimate the percentage of cases of pseudohyperkalemia and pseudonormokalemia in our hospital.

Materials and methods

Individuals diagnosed with essential thrombocytosis (2010-2019) based on the WHO criteria for the classification of myeloid neoplasms and acute leukemia were considered.

The cut-off value for the interference of platelet count on serum potassium results was calculated using the

reference change value. Sensitivity and specificity were calculated using a ROC-curve, and the size of the effect by the Cohen's d.

The clinical impact of both phenomena was assessed by reviewing the medical records of individuals classified as such, and also looking for potential cases in 2019 on the laboratory information system.

Results

Fifty-four individuals with essential thrombocytosis were included. Potassium concentration correlated with platelet count (P-value<0.001; Spearman's ρ =0.394) in serum. The cut-off value of platelet count interfering potassium was 598x10^3/µL [CI95%: 533–662x10^3/µL], with an associated sensitivity and specificity of 0.67 [CI95%:0.52–0.80] and 0.58 [CI95%:0.42–0.72] respectively.

The medical records of patients classified as pseudohyperkalemia or pseudonormokalemia did not include any medical action for the modification of potassium levels. In 2019, up to 0.14% of the total serum potassium determinations were susceptible to be pseudohyperkalemia or pseudonormokalemia.

Conclusion

This study provides a cut-off value for platelet count interfering serum potassium concentrations, and brings to light not only pseudohyperkalemia-related issues, but also the pseudonormokalemia phenomenon, which usually goes unnoticed.

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INTRODUCTION

Potassium ion (K^+) concentrations in plasma are kept within a narrow range thanks to its homeostatic mechanisms. The increase *in vivo* in extracellular potassium ion (K^+), known as hyperkalemia (HK) (decrease in K^+ removal by the kidney, rhabdomyolysis, tumor lysis, hemolysis, etc.) may produce neuromuscular and cardiac hyperexcitability, resulting in mild muscle cramps, weakness, paralysis or extremely severe arrhythmia.[1,2]

Pseudohyperkalemia (PHK) is a common finding in clinical samples from individuals with essential thrombocytosis (ET) or reactive thrombocytosis (RT), as a consequence of marked in vitro elevation of serum K^+ levels in the absence of clinical evidence of electrolytic imbalances. Multiple studies have reported that this elevation may be a result of the lysis of platelets or other cell components during blood coagulation.[3-5] and has been historically defined as an increase in serum K^+ concentration of 0.4 mmol/L over plasma.[6] In contrast, pseudonormokalemia (PNK) is a less known phenomenon, although resulting from the same mechanism that goes more easily unnoticed by physicians. In PNK, where K^+ values are accepted as 'normal' when the patient is actually hypokalemic and may need to be treated.

From a preanalytical point of view, it is accepted that sample collection and management are crucial for a proper assessment of K^+ levels. Some frequent causes of PHK include an excessive duration of transportation and subsequent delay in centrifugation, sample refrigeration before centrifugation, improper phlebotomy technique, sample contamination with potassium EDTA, sample transportation through pneumatic tube, hemolysis and also possible seasonal variations in environmental temperature.[7-9] When PHK or PNK is suspected, the determination of K^+ concentration is recommended either in lithium heparin (LH) plasma or in whole blood samples with balanced LH.[10] In some countries, or specialized clinical settings, lithium heparin tubes are strongly recommended to robustly assess and rule out possible cases of falsely increased potassium values. This

requires physicians and nurses to be aware of such potential situations, and prevent any delay in the diagnosis.

Despite extensive literature evidencing a positive correlation between thrombocytosis and K^+ concentration measured in serum, very few studies have tried to establish a valid cut-off value for the platelet count above which K^+ results should be interpreted with caution, let alone their clinical impact.

Hence, the aims of our study were: 1) to establish the cut-off value for platelet count in whole blood that yields K^+ variations in serum above the reference change value (RCV) and, based on these results, 2) to estimate the percentage of cases of PHK, PNK and HK in our hospital together with the clinical outcomes related to over-or undertreatment.

MATERIAL AND METHODS

Study design

This was a retrospective observational study performed at Hospital Universitari Son Espases (Palma de Mallorca, Spain), which is a tertiary care hospital giving direct service to a population of about 325,000 inhabitants. The analytical results included in the study were obtained from the laboratory information system (LIS) GestLab (Indra Cointec, Spain), and the medical records were obtained from the hospital information system Millennium (Cerner Corporation, USA), after obtaining the approval by the Ethics Board of our institution [Research Ethics Committee of the Balearic Islands (CEI-IB), nº IB 4191/20 PI].

Patients diagnosed with ET (2010-2019) based on the WHO criteria for the classification of myeloid neoplasms and acute leukemia,[11] which were appointed for a control blood examination (including complete blood count and basic metabolic panel) by the Department of Hematology were considered. Individuals were included if they had at least two control blood analyses after diagnosis date within a time frame of <4 months, both of them including platelet count, red blood cell count ($<5.8x10^6$/uL) and leukocyte count ($<20x10^3$/uL) in whole blood, and creatinine (<1.2 mg/dL; 106.1 µmol/L), potassium and hemolysis index (≤0.003 g/dL free Hb) in serum.

Another inclusion criterion was that one of both blood analyses had a platelet count between 300-$400x10^3$/µL (reference interval: 150-$400x10^3$/µL) whereas the other had an altered result ($>400x10^3$/µL). For each patient, there was a sample with a normal platelet count and, at least, a sample with an abnormal platelet count. However, there were some patients with more than one sample with an abnormal platelet count.

Patients with chronic kidney disease, gastrointestinal disease or under treatment with drugs potentially altering potassium homeostasis were excluded (including anti-platelet therapy).

All samples were collected into tubes containing potassium EDTA (whole blood) or serum separator gel (Vacutainer, Becton Dickinson) and received at the laboratory through pneumatic tube. According to laboratory protocols, serum samples were centrifuged at 1500g for 10 min after clot formation.

All biochemical parameters were analyzed within the first hour after blood extraction on an Architect c16000 platform (Abbott Diagnostics). The reference interval for serum K^+ used in our laboratory is 3.6–5.3 mmol/L. Hematological parameters were measured by Hematology Analyzer (Abbott Diagnostics), and the analytical imprecision of the platelet count was 3.02%.

Data analysis

Basal K^+ concentration (A) was defined as the K^+ result with a platelet count within the reference range, while the false K^+ concentration (B) is

referred to the result in the sample with a platelet count above the upper reference limit.

The dependence of K^+ results on the platelet count was assessed by the representation of the percentage variation of K^+ [((B - A)/A) x 100] against the altered platelet result. For individuals with more than 2 samples (more than one with an abnormal platelet count), all differences were referred to the sample with a normal platelet count. All values were included in the analysis.

Statistical analysis

All statistical calculations were performed on the SPSS v.24 software (IBM Corporation, USA). The Kolmogorov-Smirnov test was used to assess distribution normality, and the correlation between variables was evaluated by means of Pearson's correlation coefficient if normally distributed and Spearman´s ρ if not. Statistical significance was set at 0.05.

The cut-off value was determined as the platelet count for which the K^+ result exceeded the RCV of our laboratory. Its related sensitivity and specificity were determined using a receiver operating characteristics (ROC) curve. RCV was calculated using the following equation:

$$RCV = 11.6\% = \pm \sqrt{2} \times Z \times \sqrt{CV_A^2 + CV_I^2}$$

where CVA is the analytical coefficient of variation in our laboratory (most adverse level: 1.3%), CVI is the intraindividual biological variation (4.8%) according to the guidelines of the Spanish Society of Laboratory Medicine (SEQC),[12] and Z is the unidirectional statistical coefficient (1.65 for 95% probability).

A statistically significant result only indicates that it is unlikely that the relationship found between variables is due to chance. However, it does not provide information about the strength of the relationship (size of the effect) or if such relationship is clinically significant. On this basis, the clinical relevance of the thrombocytosis interference was quantified by the effect size estimates using the Cohen's d (parameter generally used to refer to the magnitude of an outcome result or to the strength of the relationship between two variables, in our case, platelet count and K^+ concentration). It was calculated by estimation of the magnitude of the difference between averages of the effect obtained by thrombocytosis (false K^+ results) compared to a control group (basal K^+ results):

$$Cohen's\ d = \frac{|mean_{basal\ K^+} - mean_{altered\ K^+}|}{pooled\ standard\ deviation}$$

Values for Cohen's d less than 0.2 indicate a very low effect, while values greater than 0.8 imply a significant effect.

Clinical consequences

After setting the cut-off value for platelet count for statistically significant interference, false K^+ results corresponding to altered platelet counts were mathematically estimated using the obtained regression, thus obtaining corrected K^+ values. Patient samples with corrected K^+<3.6 mmol/L were classified as PNK episodes, those samples with 3.6≤K^+≤5.3 mmol/L (corrected values within reference interval) were classified as PHK and those with corrected K^+>5.3 mmol/L were classified as true HK.

The clinical scope of this alteration of K^+ results was assessed in two branches.

First, a review of the medical records of the individuals previously identified as cases of PNK, PHK and HK was conducted, aiming to classify clinical outcomes of such actions for the patient. Medical actions aiming to correct potassium were searched, as well as adverse outcomes of over- or undertreatment.

Secondly, a retrospective search was performed for serum K^+ results representing susceptible episodes of PNK or PHK for year 2019. The following filters were applied: K^+ = 3.6–4.7 mmol/L (for PNK) or K^+>5.3 mmol/L (for PHK); platelet count above the established cut-off value; red blood cell count (<5.8x10^6/µL); leukocyte count <20x10^3/uL; serum creatinine <106.1 µmol/L and hemolysis index ≤0.003 g/dL free Hb.

RESULTS

Fifty-four patients with ET met all inclusion criteria for our study, with a total of 94 results. The main characteristics of patients are shown on Table 1. The correlation between the percentage variation of serum K^+ and the platelet count in whole blood was statistically significant (P-value <0.001). These variables were found to be associated in a linear manner, following the equation:

$$\Delta K^+, [\%] = -2.16 + 2.3x10^{-5} \cdot \text{platelet count } [\mu L^{-1}]$$
$$\text{Spearman's } \rho = 0.394$$

The application of the RCV in the obtained equation yielded a cut-off value for platelet count of 598x10^3/µL [CI 95%: 533–662x10^3/µL] for the definition of interference, with an associated sensitivity of 0.67 [CI 95%: 0.52–0.80] and specificity of 0.58 [CI 95%: 0.42–0.72] (Figure 1). No relationship was found between potassium variation and mean platelet volume (MPV).

The calculation of Cohen's d for the estimation of the size effect of thrombocytosis on the K^+ values yielded d =1.0.

For the correction of serum K^+ results, the established cut-off was used. Of all corrected values, 6.5% (n=7) corresponded to PHK episodes and 1.9% (n=2) to PNK episodes. No results reflecting true HK were found.

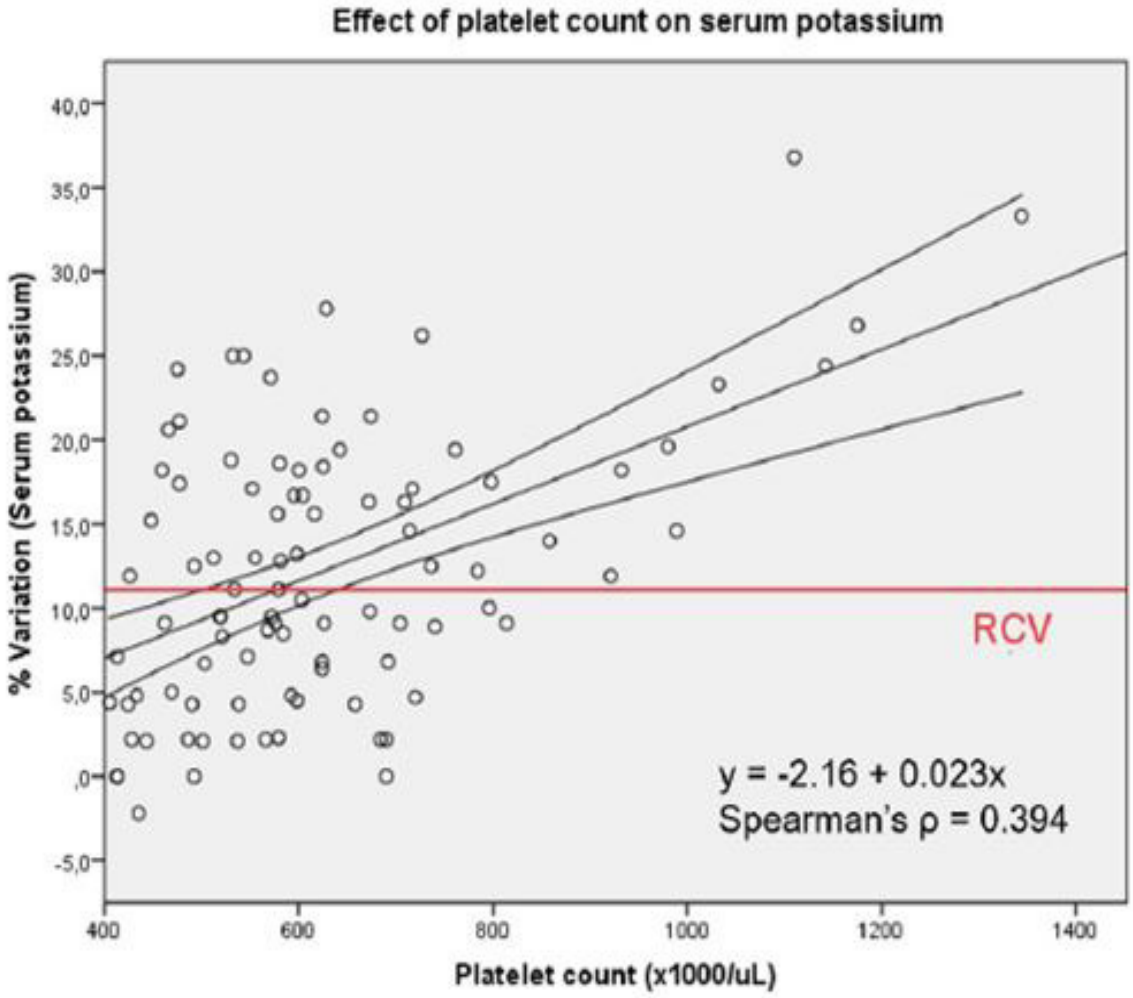
The medical records associated with these 9 episodes did not include any medical action for the modification of K^+ levels. In addition, patients with PNK did not show any adverse clinical evidence for the lack of treatment. Likewise, as a result of the second study, we found out a total of 430 results susceptible of being PNK and 75 of PHK for year 2019 (of over 368,000 K^+ serum tests). This means that 0.14% of the K^+ results may have been incorrectly interpreted in case of thrombocytosis.

Table 1 **Characteristics of patients with ET (samples with an abnormal platelet count)**

	Age	K^+ (before correction), n_R*		Classification (after correction), n_R			Platelet count
		Normokalemia	Hyperkalemia	PNK	PHK	HK	
ET (n=54)	63 [22 – 95]	4.7 [4.5–4.9], 87	5.6 [5.4–5.8], 7	2	7	-	592 [509–719] x10^3/µL

n_R: number of results*
Data median [min-max]. Age
Data median [IQR, interquartile range]. Platelet Count; K+ (mmol /L)

Figure 1 **Impact of platelet count on serum K+ measurements regarding to the baseline values**



DISCUSSION

The retrospective analysis of patients with ET allowed us to verify the dependence of in vitro serum K^+ with platelet count, as previously reported elsewhere.[13]

There is scarce literature regarding the establishment of a cut-off value above which serum K^+ is significantly interfered by thrombocytosis, and the available cutoff values are all based on a fixed change in K^+, independently of its baseline concentration. Thus, Thurlow et al[7] and Ranjitkar et al[14] reported that platelet counts above $500x10^3/\mu L$ cause a variation in K^+ of >0.5 mmol/L. In our study, exclusion of hospitalized individuals was considered, thus assuring that patients with kidney disease, under fluid therapy or under treatment with other drugs potentially altering K^+ homeostasis would not affect the findings.

Moreover, a new feature of our approach was that only effects above the reference range were included in the study (this explains why basal platelet counts were chosen close to the upper limit of reference), leading to a higher robustness of the conclusions. In our opinion, the abovementioned cut-off values could be optimized more accurately since, given the RCV in our laboratory, for individuals with basal K^+ <4.0 mmol/L variations below 0.5 mmol/L could be significant, whereas for individuals with basal K^+ >5.0 mmol/L, variations >0.5 mmol/L would be needed for thrombocytosis to be considered responsible for the increase.

The application of the abovementioned exclusion criteria for individuals diagnosed with ET allowed excluding results potentially generating a bias in the statistical analysis, hence optimizing the selection of data from previous studies.[7,14] In addition, considering the individuality index (II) of serum K^+ (II = 1.0 according to the Biological Variation Database of the European Federation of Clinical Chemistry and Laboratory Medicine, EFLM),[15] the RCV was considered as the statistical element to establish the magnitude of interference.

Regarding the statistical results, the coefficient of correlation obtained for the regression is in accordance with related studies, although the sensitivity associated with the cut-off obtained in our study is lower than that reported by Thurlow et al,[7] probably due to the dispersion of our results.

The clinical relevance of thrombocytosis in the determination of serum K^+ is evidenced by the result of Cohen's d coefficient.-

Pseudohyperkalemia is a finding well known by physicians attending individuals with ET, which is brought to light by the absence of unnecessary clinical interventions in the medical records reviewed in our study. Nevertheless, a professional unfamiliar with this condition may be confused by elevated potassium levels without accompanying reports on hemolysis. This may result in unnecessary treatments leading to potentially dangerous outcomes such as iatrogenic hypokalemia.[16] These observations point out that laboratory reports should include caution notifications if PHK is suspected. Correction of K^+ results in cases of possible PHK could also be a useful alternative to avoid overtreatment, although further studies need to be performed in this direction. Similar strategies have also been suggested for PHK due to hemolysis.[14,17]

Regarding pseudonormokalemia, to the best of our knowledge, this is the first study assessing this phenomenon in clinical samples. Although in our study no cases of moderate to severe hypokalemia were found, the lack of knowledge of this situation and the lack of warnings could increase the risk of underestimation with potential adverse clinical outcomes.

The retrospective study using our cutoff value for cases susceptible of PHK and PNK in our population offers a broad vision of the scope of the interference and the magnitude of potential repercussions being in our case, particularly remarkable the susceptible episodes of PHK. In our study, 8 of the results show moderate (K^+= 6.1–6.5 mmol/L) or severe HK (K^+ >6.5 mmol/L), which means a high probability of carrying out a medical action if the platelet count has not been considered. Therefore, it would be interesting to add such warnings as an aid for the physician towards an improvement in patient safety. Nevertheless, prospective studies comparing serum and plasma samples in patients with thrombocytosis are still needed for the verification and validation of our results.

Translation of research into routine laboratory practice is fundamental. The first steps for the application of our findings should be to include algorithms into the LIS, so that potentially interfered potassium values appear with a comment

and/or those results are held for specialist review. Alternative strategies could be to request the determination of K^+ concentration either in lithium heparin (LH) plasma or in whole blood samples with balanced LH to confirm the presence of the interference.[10]

This study has some limitations, mainly related with its retrospective nature and the trust in the records from the laboratory and hospital information systems. A larger sample size for the establishment of a cut-off value for the platelet count would add robustness to our conclusions, as the dispersion of our results leads to a low coefficient of correlation. This could also be partially explained by the interindividual biological variability, which was not included as variable in this study. Besides, result correction should always be performed with care and potential misclassifications should be studied. The decision on using plasma samples instead of serum in the measurement of potassium lays in each laboratory, depending on their organization and the distance of blood collection points. In addition, other unusual potential sources of contamination, such as EDTA contamination, were not specifically tested.

As strengths, this study provides an improved cut-off value by specifically selecting the participants: platelet counts homogeneously distributed in the pathological region above the upper limit of reference and avoiding hemolyzed samples. In addition, this study brings to light not only PHK-related issues and its overtreatment consequences, but also the PNK phenomenon, which may usually go unnoticed and whose lack of treatment might carry adverse outcomes for the patient. Thus, as individuals with platelet counts above the cutoff value could present potassium values exceeding the RCV, those results should be interpreted cautiously. Although the authors are aware that our cutoff value is not optimal, they consider it is of a great clinical usefulness, given that a platelet count above such cutoff value leads to a significant (false) increase in potassium values, with subsequent potentially adverse medical actions. Future studies warrant a better adjustment and optimization of the regression and cut-off values.

In conclusion, and considering hypo- and HK as life-threatening disorders, with an essential early detection and treatment, laboratory professionals need to identify possible interferents and remove or minimize them, so that the K^+ result on the laboratory report is accurate. This will allow physicians to take medical actions according to the real needs of the patient and avoid under- or overtreatments, thus enhancing patient safety.

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Declaration of competing interests

All authors declare no conflicts of interest.

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Ethics approval and consent to participate

The study was approved by the Ethics Board of our institution [Research Ethics Committee of the Balearic Islands (CEI-IB), nº IB 4191/20 PI].

Contributorship

Study conception and design: JAD, DMG, BL, JMB; acquisition of data: JAD, MAB, SAJ; analysis and interpretation of data: JAD, JMB; drafting of manuscript: JAD, JMB, BL, EMM. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

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