American Journal of Emergency Medicine 33 (2015) 1152–1157

Contents lists available at ScienceDirect

American Journal of Emergency Medicine

journal homepage: www.elsevier.com/locate/ajem

Original Contribution

Complete blood count at the ED: preanalytic variables for hemoglobin and leukocytes☆

Paolo Carraro, MD [a,*], Gianna Vettore, MD [b], Andrea Padoan, PhD [a], Elisa Piva, MD [a], Mario Plebani, MD [a]

[a] Department of Laboratory Medicine, Padova University Hospital, 35100, Padova, Italy
[b] Emergency Department, Padova University Hospital, 35100, Padova, Italy

ARTICLE INFO

Article history:
Received 31 December 2014
Received in revised form 27 April 2015
Accepted 13 May 2015

ABSTRACT

Objective: The objective of this study is to determine the ways in which preanalytic factors related to physiologic status can affect the complete blood cell count (CBC) in patients referring to an emergency department (ED).
Methods: Over a 1-year period, the results of hemoglobin (Hb) level and white blood cell (WBC) counts of the first CBC tests undertaken in consecutive patients (n = 11 487) referring to the ED were compared with those obtained in the same patients at a second test undertaken within 24 hours of admission. A prospective evaluation of the same differences was made in another group (group 2) of 1025 consecutive ED patients, several clinical characteristics being taken into consideration.
Results: Mean Hb concentrations were higher in the first (range, 8.0-15.9 g/dL) than in the second test results (median overestimation, 0.4-0.8 g/dL; $P < .0001$). At multivariate analysis of results in group 2 patients, fluid administration (>0.5 L) and the presence of edema played a significant role in the initial overestimation of Hb level ($P = .001$ and $P = .045$, respectively). The comparison between leukocyte counts (WBC) showed that values from the first were higher than those in the second test (median overestimation ranging from 0.42 to 3.63 × 10^9/L cells, in the range counts from 4.0 to 30.0 × 10^9/L). None of the clinical factors studied appeared to have affected this overestimation.
Conclusions: On interpreting CBC results in patients admitted to the ED, physicians must consider the effect of physiologic variables on Hb level (mainly hydration status) and WBC count (mental and physical stress).

1. Introduction

As recently demonstrated, errors in test requesting (appropriateness) and interpretation (competence) are major sources of diagnostic errors [1], and it has long been known that preanalytic conditions significantly affect intraindividual fluctuations, impacting on the results of complete blood count (CBC), one of the most commonly requested laboratory tests. Conditions such as preparation of the patient, fasting, the patient's position before and during phlebotomy, the use of tourniquets during phlebotomy, the time of day or night when venipuncture is performed, physiologic changes, and circadian rhythm affect test results. In their exhaustive review appearing in 1977, Statland and Winkel [2] summarized the effects of factors influencing CBC results.

On admission to the emergency department (ED), samples are often taken in the afternoon or at night from patients who may not have fasted, may have exercised, smoked tobacco, and/or consumed alcohol, tea, or coffee. These patients may be seen by an emergency physician after a long wait and/or after being in an orthostatic or supine position for several hours; they are also sometimes under intense psychologic stress. The physician must bear these factors in mind when comparing results obtained in an emergency setting with the reference values, which are usually obtained in subjects under standard, ideal conditions [3]. Laboratory staff members are often asked by the physician to interpret the results or even undertake retesting and, in the space of a few hours, to recheck the results when significant differences in the cited variables are found in the same individual. In the past, the most commonly investigated preanalytic variable was posture, and the first study to investigate its influence, by Thompson et al [4], appeared in 1928. The authors found that the act of standing upright increased hydrostatic pressure in the lower limbs and could lead to a loss of plasma volumes. Youmans et al [5] estimated that the incidence of this alteration ranged from 6% to 15% in healthy subjects. More recently, Jacob et al [6] referred to the condition observed after hospital admission in patients who undergo phlebotomy in a supine position as "postural pseudo-anemia." In their experimental study on 28 healthy subjects, the authors found that the average hematocrit was 41.8% when samples were taken from the patient in an upright position against 37.7% in a supine position [6,7]. Rapid changes in white blood cell (WBC) count, with an increase in leukocytes, mainly neutrophils, can be caused by inflammatory diseases and psychologic stress from pain, anxiety, or trauma. The impact of psychologic stress on the distribution of leukocytes was

☆ The authors neither have had financial support to disclose nor have they any potentially competing interests.

* Corresponding author at: Laboratorio di Patologia Clinica, Ospedale Sant'Antonio, Via Facciolati 71, 35128 Padova, Italy. Tel.: +39 049 8216 612; fax: +39 049 8216 614.
E-mail address: paolo.carraro@sanita.padova.it (P. Carraro).

http://dx.doi.org/10.1016/j.ajem.2015.05.011
0735-6757/

first reported by Mora et al [8] in 1926 and later confirmed in experimental studies by Wittkower [9] and Toft et al [10]. In 1951, Samuels [11] observed a case of early polymorphonucleated leukocytosis in response to adrenaline, with a peak at 2 to 4 hours; this finding was confirmed by Benschop et al [12]. Cortisol has also been found to play a role in the acute onset of leukocytosis in patients presenting in the ED [13]. However, no studies have been conducted to gain further insight on the true extent of the phenomenon and the impact it may have on decision making in the ED setting. Moreover, the exact degree of variation, particularly for hemoglobin (Hb) level and total WBC, has not been reported in the literature.

To better understand the nature and severity of variability with a view to improving upon the interpretation of laboratory data in the context of the ED, over a 1-year period (from July 1, 2011, to June 30, 2012), we conducted a retrospective study based on the collection of data from a large group of patients consisting of all consecutive subjects (n = 11 487, group 1) for whom CBC had been requested on admission to ED and data from a second WBC evaluation made within 24 hours after the first. To obtain detailed information regarding the specific variables that may affect WBC and Hb level results and their interpretation, we made a prospective analysis of data in a second group of consecutive patients admitted to the ED (n = 1025, group 2), who underwent 2 CBC tests within the first 24 hours, by collecting, recording, and investigating several clinical parameters.

2. Methods

2.1. Study setting

The University-Hospital of Padova, a health care institution with 1600 beds, is a teaching hospital and research center of national and international relevance, with a catchment population of more than 450 000. In the emergency care setting, the total number of adult patients referring annually from Padova city and its surrounding area is about 120 000, of which 25 000 (21%) are hospitalized. The Department of Laboratory Medicine includes an emergency section; a core laboratory for clinical chemistry, hematology, coagulation, autoimmune serology, and immunology; and specialized sections in molecular biology, clinical proteomics, and newborn screening. All laboratory tests requested as urgent (STAT) by the physicians of ED and hospital wards are performed in the emergency section, whereas all the nonemergency analyses for the wards are performed in the core laboratory.

2.2. Patients

In a 1-year period, from July 1, 2011, to June 30, 2012, the ED requested a total of 40 832 CBCs, all of which were performed by the emergency section using 2 Advia 2120i analyzers (Siemens Healthcare, Milan, Italy). In 11 487 cases (group 1), CBC analysis was followed by a second test within 24 hours, performed in the same emergency section of the laboratory or in the core laboratory. In view of the high-throughput characteristics of the core laboratory, all tests were performed using an automation system Sysmex HST equipped with 3 XE2100 instruments (Dasit, Milan, Italy). In a second group of patients (group 2), 1025 consecutive ED admissions were evaluated to ascertain whether any variable because of treatment might explain the differences identified in the overall population (group 1); we conducted a prospective analysis of 2 successive CBCs, determinations being made within 24 hours. Clinical data were obtained from the ED reports in the patients' electronic records. The data included patients' condition observed during the examination: body temperature (considered positive if >37.5°C), significant pretibial pitting edema, and use of antibiotics and antineoplastic drugs; some treatments performed at the ED (blood transfusion, systemic cortisone, diuretics, antibiotics, volume of fluid infusion) were also recorded. The patients' identities were promptly removed to ensure anonymity.

2.3. CBC methods and performances

The CBCs were performed within 4 hours of collection in tubes containing EDTA. Both hematology analyzers run on flow cytometry and use a routine photometric method for Hb analysis, but they differ in the method used for the leukocyte and differential count. The ADVIA 2120i method is based on light scatter and cytochemistry, whereas that of Sysmex XE-2100 on light scatter, impedance, and fluorescence. To assure the accurate alignment of all instruments (in addition to routine quality control procedures), the laboratory used 3 specific strategies: in each section (core laboratory and emergency), 5 routine fresh samples and specific difference limits based on biologic variations (maximum acceptable gap of 4.1% for Hb level and 14.6% for WBC count) [14,15] for data comparison; a preliminary identification of 1 instrument as a reference analyzer was made by the 2 sections of the laboratory participating in the same external quality assessment (EQA) scheme, with regular comparison of results; every 6 months, all instruments were aligned by measuring 25 fresh blood samples. During the study period, any differences were corrected by means of instrumentation maintenance procedures; systematic correction of the calibration of the 5 instruments used was not necessary. In estimating the effect of preanalytic variables, we calculated all changes as differences of the first minus the second CBC test obtained within 24 hours after the first test. It was assumed that the results of this second test evidenced the patient's condition in a more stable situation, usually during the following morning, after fasting, rehydration, and, in some cases, relief from pain. However, we were unable to identify the real posture and the degree of stress of all patients at the time of the blood sampling.

2.4. Statistical analysis

Changes in Hb level and WBC count after admission to the ED, divided into concentration classes, were reported in median and interquartile ranges. Overall, differences in results from the first and those from the second CBC examinations were evaluated by the Wilcoxon matched pair test. To meet the normality assumption, multivariate analysis of Hb level differences between the second and the first test was made after square transformation of variables. Repeated measures analysis of variance was used to determine whether the difference in Hb level in the 2 consecutive measurements was associated with age, sex, fever, edema, and/or fluid administration, included in the model as covariates. The nonparametric test across ordered groups was used to assess whether fluid administration in increasing amounts was associated with differences in Hb level. Because WBC count differences between the first and the second examination did not meet the normality assumption, even after transformation, we used a different approach for WBC analysis, using the nonparametric Kruskal-Wallis test. Statistical analyses were made using STATA 13.1 (StataCorp, Lakeway, TX).

3. Results

3.1. Analyzers performances

The first issue considered was the efficiency of the alignment procedures of hematologic analyzers. The results from the EQA scheme during the study period are presented in Table 1; the bias between the 2 sections of the laboratory was obtained from these results. For all values considered, the bias ranged from −2% to +2% for Hb level and −6.0% to +3.9% for WBC count. These differences are lower than the optimal allowable total error limits (TEa), as calculated based on the biologic variation, which are 2.06% for Hb level and 7.30% for WBC count [14,15].

Concerning Hb level and WBC count imprecision during the study period, the coefficient variation values, calculated using the internal quality control data, ranged from 0.8% to 1% for Hb level and from 1.72% to 2.96% for WBC count, in line with the desirable analytic goals based on intraindividual variation [14,15].