Clinica Chimica Acta 433 (2014) 145–149

Contents lists available at ScienceDirect

Clinica Chimica Acta

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

Development of a new point-of-care testing system for measuring white blood cell and C-reactive protein levels in whole blood samples

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ARTICLE INFO

Article history:
Received 18 November 2013
Received in revised form 31 January 2014
Accepted 7 March 2014
Available online 15 March 2014

Keywords:
CRP
Granulocyte esterase
Inflammation
Immunochromatographic assay
POCT
WBC
Leukocyte

ABSTRACT

Background: White blood cell (WBC) count and C-reactive protein (CRP) level are the most common markers of inflammation. There is a growing need for point-of-care testing (POCT) of WBC and CRP, and more advances in convenient devices are required. We developed an analyzer-free POCT system for measuring WBC and CRP using a low volume blood sample.
Methods: The POCT-WBC is based on the granulocyte esterase assay, while the POCT-CRP is based on the immunochromatographic assay. These kits were examined for precision as well as correlation with currently used popular commercial automated assays. The correlations were clinically analyzed in children with acute infection ($n = 62$; mean age 4.2 y). The correlations regarding the monitoring of values were further examined in several follow-up subjects.
Results: The POCT-WBC and POCT-CRP kits demonstrated good precision. POCT-WBC exhibited a significantly close correlation with those of the control assay ($r = 0.94$, $p < 0.05$). The results of POCT-CRP also exhibited a significantly close correlation with those of the control assay ($r = 0.94$, $p < 0.05$). In the follow-up study, the results of the respective kits were similar to those of the control assays.
Conclusions: The POCT-WBC and POCT-CRP are promising tools for assessing infection in clinical practice.

1. Introduction

White blood cell (WBC) and C-reactive protein (CRP) levels are the most commonly used blood measurements in the diagnosis and therapeutic monitoring of inflammation [1–4]. Patients with acute infection are frequently seen in a variety of settings (e.g., not only hospitals, but also primary care, home care, rural, and general practitioner facilities). Quick medical decision-making is required when treating patients with acute infection, and not all facilities maintain the machines required to measure promptly WBC and CRP [3–7]. Much attention has recently been paid to point-of-care testing (POCT) using portable, table-top, handheld and/or disposable devices operated in non-laboratory environments by non-technical staff to obtain rapid on-site results [7]. There is a growing need for POCT of WBC and CRP [2–5,8].

Some POCT devices for measuring CRP are currently available [2,4,8–12]. Among these devices, clinical studies have been conducted using the QuikREAD test [4,6,13–17] and Nycocard test [15,17–20], in particular. Most clinical studies have indicated relevant contributions of these tests to assessing inflammation in clinical practice [6,16–20]. However, these tests generally require the additional use of specific analyzers (even though the analyzers are small relative to the machines as used in hospitals), the samples must be pretreated before measurement in some tests [12], or the reaction after measurement must be checked visually due to the principle of latex agglutination reaction in some tests [9,13]. Although the development of POCT kits for WBC may be behind that of POCT devices for CRP or hemoglobin [3], some POCT devices for WBC, for instance, the HemoCue WBC test [21] and QBC Star test [22], are currently available [3,7,9]. However, no clinical studies of these tests have been reported, and the tests also require the use of specific analyzers. Thus, further advances in the POCT devices for CRP and WBC are still required.

Another important point regarding the assessment of inflammation in clinical practice is the simultaneous measurement of WBC and CRP, as these measures have somewhat different characteristics as markers [9]. The WBC level tends to increase during the superacute phase of inflammation, while the CRP level increases in the later stage [1,9,23]. The simultaneous determination of both markers can aid in understanding the phase of inflammation. Furthermore, the increase in WBC during the acute phase depends on that of granulocytes, primarily neutrophils [2], and the tests reflecting of the level of granulocytes (neutrophils) can influence the administration of antibiotics, regardless of the CRP level, since an increase in neutrophils is traditionally considered to be suggestive of bacterial infection [24].

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http://dx.doi.org/10.1016/j.cca.2014.03.004
0009-8981/

2. Materials and methods

2.1. POCT-WBC and hs-cRP

Details of the principles and procedures of these assays are available from the authors. The results of POCT-WBC are classified into one of three grades: (1+) for a WBC level of < 10000/μl, (2+) for a WBC level of 10000 to 15000/μl, and (3+) for a WBC level of ≥ 15000/μl (the WBC levels are based on a cut-off level for clinical evaluation [25]). The results of POCT-CRP are classified into one of three grades: (−) for a CRP level of < 0.6 mg/dl, (1+) for a CRP level of 0.6 to 5 mg/dl, and (2+) for a CRP level of ≥ 5 mg/dl.

2.2. Precision of POCT-WBC and POCT-CRP

The precision of the POCT-WBC and POCT-CRP kits was evaluated for with-in run, between-run and interobserver precision, using blood samples with known concentrations. As for the POCT-WBC, the blood samples were prepared with 3200/μl, 7000/μl and 13,000/μl of neutrophils, respectively. The concentrations of neutrophils were determined with a widely approved automated blood cell analyzer (Horiba Co. Ltd.). As for the POCT-CRP, the blood samples were prepared with 0, 0.6 and 5 mg/dl of CRP, respectively. The concentrations of CRP were determined based on the standardized materials certified by the code of ERM-DA474/ the International Federation of Clinical Chemistry. We tested 10 replicates at each WBC (neutrophil) or CRP level.

2.3. Correlation between the results of control assays and those of POCT-WBC or POCT-CRP kit

The WBC level measured using the POCT-WBC was compared with that measured using an automated blood cell analyzer (Coulter LH500; Beckman Coulter, Inc.); this analyzer uses the electric resistance method [25]. The percentages of neutrophils were also determined using this analyzer in some cases. The CRP level measured using the POCT-CRP was compared with that measured with a reagent (Dade Behring. Diagnostics, Inc.) using an automated analyzer (7080; Hitachi High-Technologies Corp.). These control assays are popularly applied in daily practice. Correlations for the respective values were observed using Spearman's rank correlation tests. Between-group differences were observed using the Mann–Whitney *U*-test. A $p < 0.05$ was considered significant.

The clinical studies were conducted using whole blood samples. First, a healthy control population without any infection ($n = 8$; mean age 36 y) was tested. Thereafter, the main study was performed in a population of children with acute infection ($n = 62$; mean age 4.2 y). The subjects visited the Jichi Medical University Hospital due to a chief complaint of a common cold or gastroenteritis, and were diagnosed with an acute infectious disease. The study was approved by the Jichi Medical University Ethics Committee, and all subjects and their parents provided written informed consent.

A portion of the subjects repeatedly provided blood samples during multiple visits to the hospital according to the course of the disease. During the follow-up period, the correlations between the levels obtained using the control assays and those obtained using the POCT-WBC and POCT-CRP were investigated.

3. Results

3.1. Precision of the POCT-WBC and POCT-CRP

Upon replication, the samples prepared with 3200/μl, 7000/μl and 13,000/μl of neutrophils always corresponded to the semi-quantitative grade of (1+), (2+) and (3+) for WBC, respectively, on the POCT-WBC. Similarly, the samples prepared with 0, 0.6, and 5 mg/dl of CRP always corresponded to the semi-quantitative grade of (−), (1+) and (2+) of CRP, respectively, on the POCT-CRP. In line with the 100% in consistency of the with-in run precision, the between-run precision for 5 consecutive days was also 100%. In addition, the interobserver precision tested by different three operators was 100%.

3.2. Correlation between the results of control assays and those of the POCT-WBC or POCT-CRP

In the study consisting of control subjects without any infection, their WBC levels measured using the control assay were all <10,000/μl, and those measured using the POCT-WBC all showed a grade of (1+). In the study of children with acute infection, the WBC level measured using the POCT-WBC corresponded to that measured using the control assay in 58 samples (94%), as shown in Table 1. Four samples showed inconsistent levels between the POCT-WBC and control assay. The WBC level measured using the POCT-WBC was significantly and closely correlated with that measured using the control assay ($r = 0.94$, $p < 0.05$; Fig. 1).

In the study consisting of control subjects without any infection, their CRP levels measured using the control assay were all <0.6 mg/dl, and those measured using the POCT-CRP all showed a grade of (−). In the study of those with acute infection, the CRP level measured using the POCT-CRP corresponded to that measured using the control assay in 56 samples (90%), as shown in Table 1. Six samples showed inconsistent levels between the POCT-CRP and control assay. The CRP level measured using the POCT-CRP was significantly and closely correlated with that measured using the control assay ($r = 0.94$, $p < 0.05$; Fig. 2).

Additionally, we observed the correlation between the levels obtained using the POCT-WBC and POCT-CRP in the study of acute infection. A weak correlation was seen in this population (Table 1).

3.3. Follow-up study of the correlation between the results of control assays and those of the POCT-WBC or POCT-CRP

In 4 subjects, we investigated blood samples at least four times during the follow-up period. The levels obtained using the POCT-WBC or POCT-CRP were similar to those obtained using the control assays (Fig. 3).

4. Discussion

Today, the WBC and CRP levels are crucial markers for the diagnosis and therapeutic monitoring of inflammatory disorders, such as infectious diseases. Under a growing need for POCT of WBC and CRP, some

Table 1
Consistency between the POCT-WBC and CRP levels against automated analyzer (control assay) and against themselves.

		WBC counts (control assay)			Total
		<10,000/μl	10,000 to 15,000/μl	≥15,000/μl	
POCT-WBC	1+	38	3	0	41
	2+	0	15	1	16
	3+	0	0	5	5
	Total	38	18	6	62
		CRP (control assay)			Total
		<0.6 mg/dl	0.6 to 5 mg/dl	≥5 mg/dl	
POCT-CRP	–	32	1	0	33
	1+	2	15	1	18
	2+	0	2	9	11
	Total	34	18	10	62
		POCT-WBC			Total
		1+	2+	3+	
POCT-CRP	–	26	11	4	41
	1+	6	5	5	16
	2+	1	2	2	5
	Total	33	18	11	62

WBC: white blood cell, POCT: point-of-care testing, CRP: C-reactive protein.