Journal of Immunological Methods 432 (2016) 82–86

Contents lists available at ScienceDirect

Journal of Immunological Methods

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

Research paper

# Distinction between bacterial and viral infections by serum measurement of human neutrophil lipocalin (HNL) and the impact of antibody selection

Zhang Yu [a], Huang Jing [b], Pan Hongtao [c], Jia Furong [c], Jin Yuting [b], Shengyuan Xu [d], Per Venge [d,*]

[a] Changchun Brother Biotech Co., Ltd., Changchun 130062, China
[b] Laboratory Department of the First Affiliated Hospital of Jilin University, Changchun 130021, China
[c] Laboratory Department of the 208th Hospital of PLA, Changchun 30000, China
[d] Department of Medical Sciences, University of Uppsala, Uppsala, Sweden

ARTICLE INFO

*Article history:*
Received 22 January 2016
Received in revised form 15 February 2016
Accepted 16 February 2016
Available online 17 February 2016

*Keywords:*
Lipocalin
Acute Infection
Diagnosis
CRP
Neutrophil

ABSTRACT

The distinction between acute infections of bacterial or viral causes is clinically important, but often very difficult even for experienced doctors. Previous studies indicated that serum measurements of HNL (Human Neutrophil Lipocalin) might be a superior diagnostic means in this regard, but also indicated that the antibody conformation of the HNL assay might have an impact on the diagnostic performance. The aim of the present report was to examine this further.

*Methods:* Several different (n = 24) HNL ELISA assays were developed using different combinations of monoclonal and polyclonal HNL antibodies. Sera were collected from healthy persons (n = 188) and from 155 patients with acute infections before any antibiotics treatment. The patients were diagnosed as having bacterial (n = 69) or viral causes (n = 86) of their infections. Plasma and serum were also examined by Western blotting using HNL-specific polyclonal antibodies.

*Results:* The optimal assay format for the distinction between bacterial and viral infection resulted in an area under the receiver operating characteristics curve (AuROC) for S-HNL of 0.98.
(95% CI 0.94–1.00) as compared to 0.83 (0.76–0.88) for blood neutrophil counts and 0.69 (0.61–0.76) for S-CRP. Results also showed that different assay formats of HNL identified monomeric and dimeric HNL differently, the monomeric HNL being elevated in viral infections and the dimeric HNL being elevated in bacterial infections.

*Conclusion:* We conclude that serum measurement of HNL is a superior diagnostic means to distinguish between acute infections caused by bacteria or virus. For optimal clinical performance the immunoassay should address conformational epitopes in the dimeric HNL.



## 1. Introduction

The distinction between acute infections of bacterial or viral causes is clinically important, but often very difficult even for experienced doctors. These difficulties often lead to unnecessary treatment with antibiotics, which is unfortunate in the light of the growing problems with antibiotics resistance (Laxminarayan et al., 2013; Nathan and Cars, 2014). Currently used tools that may aid in the distinction are mainly white blood cell counts and CRP (Hopstaken et al., 2003; Andreeva and Melbye, 2014), whereas other recent biomarkers such as procalcitonin and CD64 are less wide spread or useful (Venge et al., 2015a). White blood cell counts and CRP, however, typically show clinical performances of 70–80% sensitivity and specificity, which means that a large proportion is misdiagnosed. The need therefore for more accurate tools is highly warranted.

HNL (human neutrophil lipocalin) was first identified in and purified from human neutrophils in the mid 90th (Xu et al., 1994). HNL was given its name, since it contains a conserved amino acid structure that is common to the large family of other lipocalins (Xu and Venge, 2000). HNL is contained in the secondary granules of human neutrophils and readily released to the extracellular environment upon stimulation. It is a fairly complicated molecule that exists in the neutrophils as a monomeric, 22 kD-protein, and as a dimeric, 45 kD-protein. A proportion is also associated to gelatinase (MMP9) of neutrophils, hence given the alternative name NGAL (neutrophil gelatinase associated lipocalin) (Kjeldsen et al., 1993). Epithelial cells may also be induced to produce HNL, but only in the monomeric form (Cai et al., 2010). In urine HNL/NGAL was shown to be elevated in patients with acute kidney injury (AKI) (Mishra et al., 2005), but also in patients having urinary tract infections (Cai et al., 2009). The distinction between these two causes with commercially

* Corresponding author at: Department of Medical Sciences University Hospital SE-751 85 Uppsala Sweden.
*E-mail address:* per.venge@medsci.uu.se (P. Venge).

http://dx.doi.org/10.1016/j.jim.2016.02.014
0022-1759/

available assays is difficult, whereas we showed previously that employing different antibody combinations that preferentially bind monomeric or dimeric HNL a distinction was made between epithelial involving and neutrophil involving disease (Martensson et al., 2012). Thus, potentially making the diagnosis of AKI more specific.

Serum-measurement of HNL most likely and preferentially reflects the activity of neutrophil granulocyte i.e. their propensity to release HNL during blood coagulation. In previous studies we showed that serum levels of HNL accurately distinguished bacterial from viral infections with sensitivities and specificities >90% (Xu et al., 1995; Bjorkqvist et al., 2004; Fjaertoft et al., 2005). Such studies were based on a sensitive radioimmunoassay, which is less convenient for clinical use. We have therefore developed a series of enzyme-linked immunosorbent assays (ELISA) based on unique monoclonal and polyclonal antibodies produced against native, dimeric HNL purified from normal human blood donors. We show in this report the importance of the antibody configurations of such assays for their clinical performance and also show the superiority of serum measurement of HNL as a diagnostic tool as compared to current procedures using white blood cell counts and CRP.

## 2. Patients and methods

Altogether 192 patients (71 females, median age 52 years IQ range 29–71 years and 121 males, median age 36 years IQ range 22–56, $p = 0.003$) were recruited from the First Affiliated Hospital of Jilin University of the Jilin province of China. 155 of these patients had not taken antibiotics before admission to the hospital. The remaining 37 patients had taken antibiotics during 1–5 days before admission. In addition blood samples were obtained from 188 healthy non-infected subjects. (males $n = 142$, mean age 25.4 years range 20–35 years, females $n = 46$, mean 26.7 years, range 22–36 years). Patients with signs and symptoms of acute infection were diagnosed as follows. The infection was likely caused by bacteria if patients had compatible clinical signs and symptoms including elevated white blood cells counts and/or elevated blood neutrophil counts and/or blood neutrophil shift to the left, body temperature > 38 °C, and effective antibiotic therapy. The bacterial infection was confirmed by positive bacterial cultures. Appendicitis was in addition diagnosed by ultrasound or CT imaging and/or examination of the removed appendix. The infection was likely caused by virus if patients had compatible signs and symptoms including normal or slightly decreased white blood cell counts and/or increased lymphocyte count. The viral infection was confirmed by positive virus serological examinations.

Blood was obtained at admittance to the hospital and the blood was allowed to coagulate for on average 2 h (range 1–3 h) at room temperature before centrifugation and collection of serum. Upper and lower respiratory tract infections were the diagnosis of 110 patients. Acute appendicitis was diagnosed in 33 patients. In the remainder of the patients ($n = 49$) various infections of the gastro-intestinal tract ($n = 22$) were diagnosed in addition to infections such as parotitis ($n = 6$) and chicken pox ($n = 9$).

Monoclonal and polyclonal antibodies against HNL were provided by Diagnostics Development, Uppsala, Sweden. Measurements in serum were performed by means of double monoclonal or polyclonal/monoclonal ELISA kits provided by Brother Biotech, Changchun, China and run according to the instructions of the provider. The between-assay imprecisions of the ELISA assays were <20% CV.

Western blotting was performed as described previously (Cai et al., 2010). Polyclonal antibodies against native, dimeric HNL were used.

## 3. Statistics

Results are presented as medians and interquartile range (IQ). Differences between groups were calculated by the non-parametric method of Mann–Whitney. p-Values <0.05 were regarded as significant differences. The clinical performances of assays were estimated by receiver operating characteristics curves (ROC-curves) and expressed as area under the receiver operating characteristics curve (AuROC), specificities and sensitivities, positive and negative likelihood ratios, positive and negative predictive values. C-statistics was applied for the comparison of AuROC-curves.

The statistical programs MedCalc Statistical Software v. 14.8.1 (MedCalc Software, Ostend, Belgium) and Statistica 64 v. 12 (Statsoft, Tulsa, OK, USA) were used.

## 4. Results

### 4.1. Clinical results

The patients with acute infections were classified as having either bacterial or viral etiology of their infections as described above. In Fig. 1a and b we show the results of HNL concentrations in serum as measured by two different assays. Only the results from patients not being treated with antibiotics are presented. One assay had the monoclonal antibody 763 as catching antibody and mab 764 as detecting antibody (ELISA

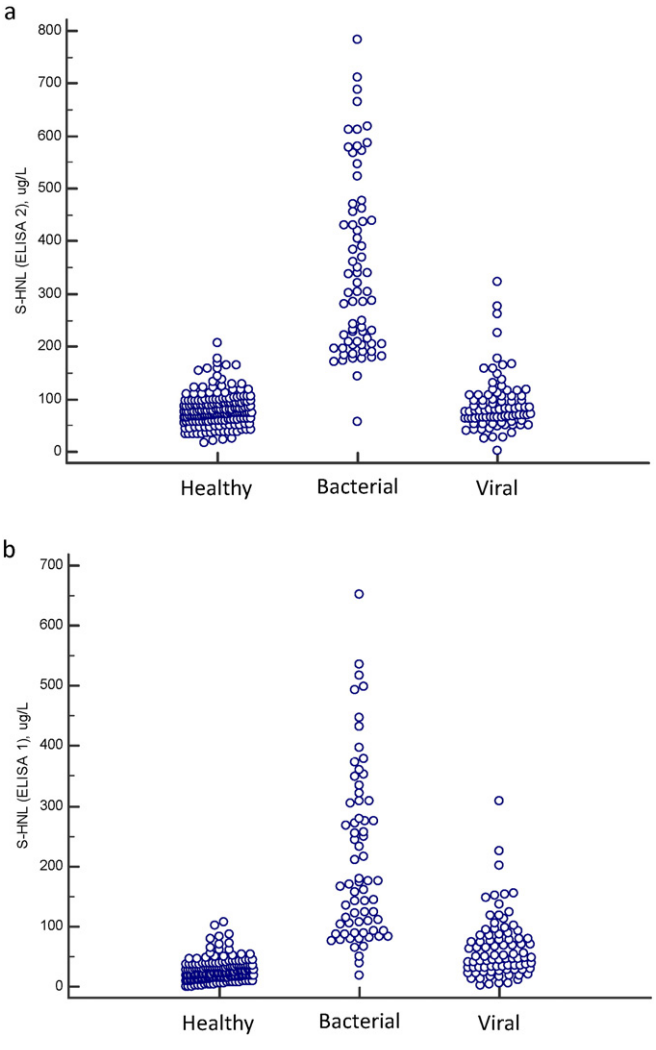


**Fig. 1.** a. HNL concentrations in serum of patients with bacterial or viral infections before treatment with antibiotics and compared to HNL concentrations in serum of healthy persons as measured by ELISA 2. The HNL concentrations in serum of patients with bacterial infections were significantly higher than in the other two groups ($p < 0.0001$) (see also Table 1). b. HNL concentrations in serum of patients with bacterial or viral infections before treatment with antibiotics and compared to HNL concentrations in serum of healthy persons as measured by ELISA 1. The HNL concentrations in serum of patients with bacterial infections were significantly higher than in the other two groups ($p < 0.001$). HNL concentrations in serum in patients with viral infections were significantly elevated as compared to serum from healthy persons ($p < 0.001$) (see also Table 1).