

# Neutrophil CD64 expression: a reliable diagnostic marker of infection in advanced cancer patients?

Giuditta Comolli<sup>1,2</sup>, Martina Torchio<sup>3</sup>, Elisa Lenta<sup>2</sup>, Benvenuto Franceschetti<sup>3</sup>, Antonella Chiesa<sup>2</sup>, Sandra A. Calarota<sup>2</sup>, Fausto Baldanti<sup>2</sup>, Luigia Scudeller<sup>4</sup>, Piero Marone<sup>2</sup>, Danova Marco<sup>3</sup>

<sup>1</sup>Laboratori di Ricerca Biotecnologie; <sup>2</sup>S.C. Microbiologia e Virologia, Fondazione IRCCS San Matteo, Pavia; <sup>3</sup>Medicina Interna e Oncologia Medica, Ospedale di Vigevano, A.O. di Pavia; <sup>4</sup>Epidemiologia Clinica e Biometria, Direzione Scientifica, Fondazione IRCCS San Matteo, Pavia, Italy

## SUMMARY

Infection and sepsis are major health problems in cancer patients. There is a need for the identification and validation of biomarkers to improve their early diagnosis and treatment. Emerging evidence showed that neutrophil CD64 is a highly sensitive and specific marker for systemic infection and sepsis in critically ill patients with various diseases but data on patients bearing solid tumors are still lacking. Using a dedicated flow cytometric assay we evaluated neutrophil CD64 expression in patients with advanced cancer without active infections to verify if it could be utilized as a reliable biomarker of early infections also in oncologic patients.

**KEY WORDS:** Infections, Sepsis, Cancer patients, Flow cytometry, Neutrophil CD64.

Received January 26, 2015

Accepted June 1, 2015

Bacterial infections are still a major cause of morbidity and mortality in patients with malignant solid tumors. In recent years, the introduction into clinical practice of new agents and new therapeutic modalities has resulted in an improvement in the prognosis for many of these patients. On the other hand, a number of new therapeutic options may adversely affect the immune system inducing a state of immunosuppression during which patients are particularly susceptible to infections, and during which their immune response to infectious episodes can be further altered. The causes of immunosuppression developing during severe bacterial infection arising in a patient with a malignant solid tumor are not yet fully known although a number of mechanisms have been

implicated. One marker that has shown particular promise as an early marker for infection is the neutrophil surface high affinity receptor for the Fc part of the immunoglobulin G (IgG) heavy chain cluster differentiation (nCD64) (Mancardi *et al.*, 2013). CD64 is a membrane-bound high-affinity receptor found on monocytes. It is, however, expressed only at low levels by neutrophils in the healthy host. Neutrophil CD64 expression corresponds to inflammatory responses during infection or tissue injury. Elevated nCD64 expression was reported in critically ill adult patients with various infections and in neonates and children with sepsis (Dimoula *et al.*, 2014; Farias *et al.*, 2014; Lyne-ma *et al.*, 2014). Regarding early diagnosis of infections, available data indicate that nCD64 seems to have high sensitivity (86%) and specificity (87%) and, even if the methodological quality of most studies is questionable (due to the limited number of patients enrolled and the utilization of different analytical approaches that hamper a comparison among studies), this marker is currently accepted in clinical practice in these patient populations (Gros *et al.*, 2012;

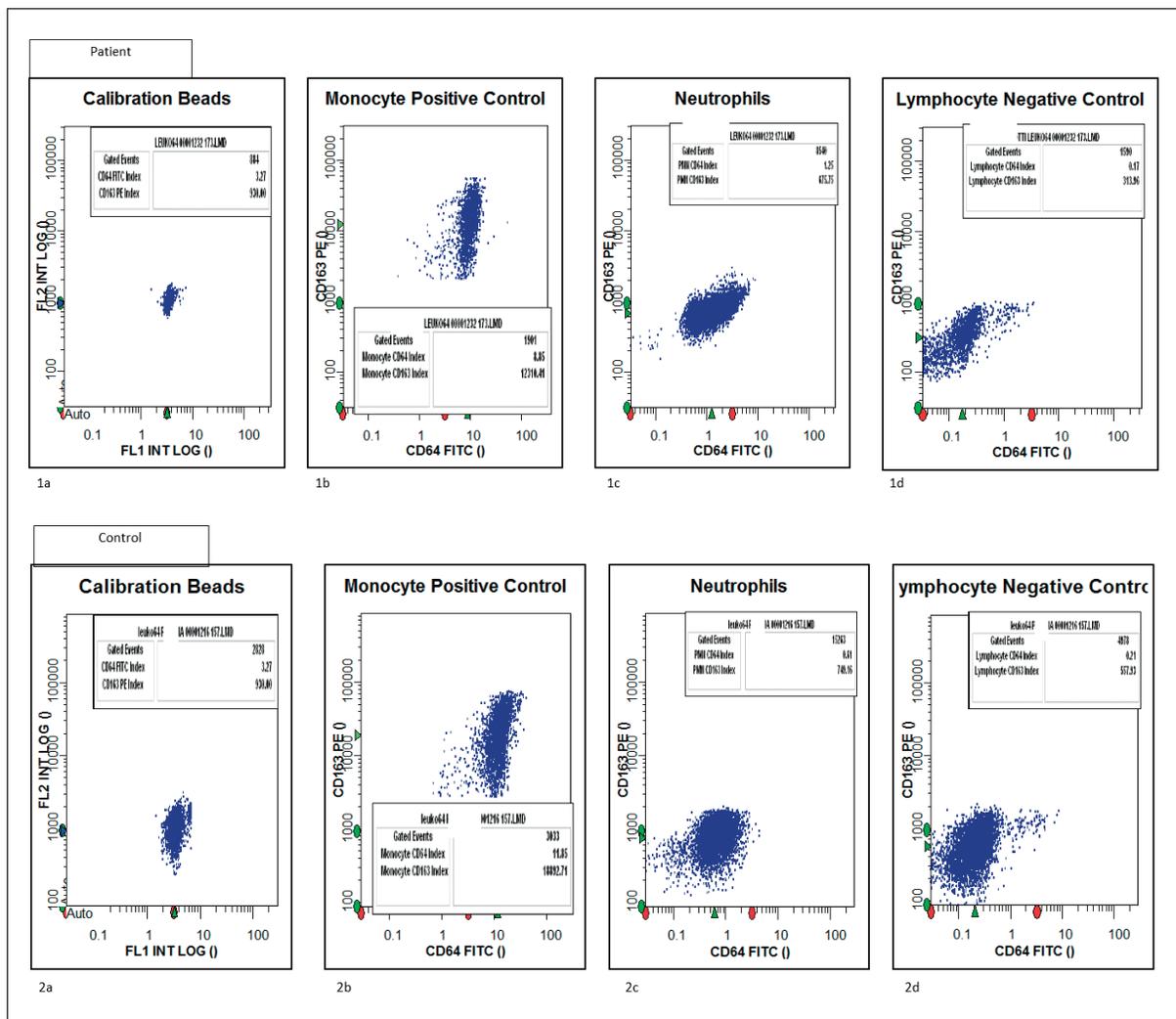
### Corresponding author

Comolli Giuditta  
Laboratori di Ricerca Biotecnologie e  
S.C. Microbiologia e Virologia  
Fondazione IRCCS San Matteo, Pavia  
Piazzale Golgi, 2 - Pavia, Italy  
E-mail: g.comolli@smatteo.pv.it

Sanquist and Wong, 2014). On the other hand, nCD64 has been shown to be upregulated in many acute and chronic inflammatory diseases as well as in several solid tumor cell lines and it has also been identified as a reliable target for immunotherapy in some human tumors (Tepfenharter *et al.*, 2009). During our ongoing study on the role of the monocyte-macrophagic system during bacterial infections and sepsis in patients with advanced solid tumors, we have focused on the nCD64 expression in patients with advanced cancer without active infections

to verify if it could be utilized as a reliable biomarker of early infections also in oncologic patients.

Consecutive adult patients admitted to Internal Medicine and Medical Oncology Unit of Pavia Hospital were enrolled in this study. The study was approved by the IRCCS San Matteo Ethics Committee and was carried out in accordance with the Declaration of Helsinki. Owing to the observational nature of this study and the fact that the blood samples used were routinely collected from all patients for the respective diag-



FIGURES 1 and 2 - Analysis of Leukocyte CD64 expression (patient Figure 1; healthy control Figure 2). Polymorpho-nucleated (PMN) CD64 expression is low with PMN CD64 Index <1,20 in healthy donors or <2,00 in hospitalized populations (Icardi *et al.*, 2009). The LeukoCD64 Assay performs simultaneous analysis of CD64 expression on lymphocytes (no expression, CD64 Index <1,00), monocytes (moderate to high expression, CD64 Index >3,00) and neutrophils (variable expression, but low in the healthy state).

nostic and therapeutic plan, the need for written informed consent was waived by the ethical review board. Three milliliters (ml) of peripheral blood were collected in EDTA-anticoagulated tubes at the time of patient admission. Samples were then analyzed within one hour using the Leuko64 Assay (Trillium Diagnostic, LLC) run on a Beckman Coulter Navios Instrument. The Leuko64 Assay is a flow cytometric assay (Wong *et al.*, 2014) that measures CD64 expression on neutrophils and serves as an *in vitro* indicator of infection/sepsis or a systemic acute inflammatory response. It currently utilizes semi-automated software that employs cluster algorithms to define cell populations reducing subjective gating decisions. The Leuko64 Assay uses a mixture of three monoclonal antibodies (MoAbs) with specificities to CD64 (clones 22 and 32) and CD163 (clone Mac2-158). The use of two Abs to different epitopes of CD64 enhances the signal to noise ratio of the assay while the addition of the MoAb to CD163, a monocyte specific Antigen (Ag), increase the specificity of leukocyte subpopulation identification.

We prospectively enrolled 28 cancer patients (10 breast, 9 colorectal, 9 lung; 20 males/8 females; median age 68 years, range 31-82 years) with advanced disease, without ongoing chemotherapy treatment and without clinical (signs, symptoms and negative clinical examination) or laboratory findings of active infection (acute and inflammatory indexes, procalcitonin, urine, sputum and blood cultures). Twenty healthy donors, matched for age and gender, were utilized as a control group. Results were expressed as a nCD64 Index as a diagnostic parameter: nCD64 Index indicated the nCD64 expression by granulocytes measured using a Leuko64 kit (Trillium Diagnostics, ME, USA) and FCM specific software. The kit includes the fluorescent beads and anti-CD64 and anti-CD163 MoAbs. The patient's sample provides an internal negative control (lymphocytes) and an internal positive control (monocytes). The CD64 Index is calculated using the ratio of the mean fluorescent intensity of the cell populations to that of the beads. Lymphocytes must have an nCD64 Index of <1 and monocytes an Index >3 for the internal controls to be considered valid. The

median of the nCD64 Index was calculated for each group. Comparison of the two groups was made using two-tailed *t* tests, and *p* value was calculated. The median values of nCD64 Index was 0.80, ranging from 0.54 to 1.15, in normal donors and 3.15, ranging from 2.68 to 4.76, in cancer patients ( $p < .05$ ), indicating that the nCD64 expression tends to be low in the healthy state and increases in patients with advanced cancer even in the absence of active infection (Figures 1 and 2).

Biomarkers have great potential to improve the diagnosis and treatment of sepsis. The available literature supports the potential utility of nCD64 as a novel diagnostic prognostic and therapeutic response (Chen *et al.*, 2014; Rogina *et al.*, 2014) in several clinical conditions. Future studies could focus on investigating the pathogenetic events that result in increased neutrophil CD64 expression in patients diagnosed with non-infectious diseases. In particular, in patients with advanced solid tumors, due to the possible cross-link between the nCD64 and the presence of the tumor itself (that leads to an abnormal nCD64 expression in cancer patients without evidence of infections), the role of the flow cytometric determination of the nCD64 expression as an early marker of infection in cancer patients needs to be better clarified before it can be routinely used in clinical practice. The future of nCD64 expression evaluation in oncological patients lies in:

- a) extensive validation studies of such molecule across different tumor types in untreated patients;
- b) possible links between its expression and the recently outlined multiple immunological effects of chemotherapy and targeted treatments (Galluzzi *et al.*, 2012; Bianchini and Gianni, 2014);
- c) the exploitation of its power in combination with other biomarkers (Sandquist and Wong, 2014).

In this field, beside the enrollment of a larger number of patients, we are currently focusing on the possible interaction of multiple surface markers such as CD64, CD40 and CD40L.

#### ACKNOWLEDGMENTS

*This study was supported by the IRCCS San Matteo grant nr. 80425 to Giuditta Comolli.*

## REFERENCES

- BIANCHINI G., GIANNI L. (2014). The immune system and response to HER2-targeted treatment in breast cancer. *Lancet Oncol.* **15**, e58-68.
- CHEN Q., SHI J., FEI A., WANG F., PAN S., WANG W. (2014). Neutrophil CD64 expression is a predictor of mortality for patients in the intensive care unit. *Int. J. Exp. Pathol.* **7**, 7806-7813.
- DIMOULA A., PRADIER O., KASSENGERA Z., DALCOMUNE D., TURKAN H., VINCENT J.L. (2014). Determinations of neutrophil CD64 expression for diagnosis and monitoring of sepsis in critically ill patients. *Clin. Infect. Dis.* **58**, 820-829.
- FARIAS M.G., DE LUCENA N.P., BÓ S.D., DE CASTRO S.M. (2014). Neutrophil CD64 expression as an important diagnostic marker of infection and sepsis in hospital patients. *J. Immunol. Methods.* **414**, 65-68.
- GALLUZZI L., SENOVILLA L., ZITVOGEL L., KROEMER G. (2012). The secret ally: immunostimulation by anti-cancer drugs. *Nat. Rev. Drug. Discov.* **11**, 215-233.
- GROS A., ROUSSEL M., SAUVADET E., GACOUIN A., MARQUÈ S., CHIMOT L., LAVOUÈ S., CAMUS C., FEST T. LE TULZO Y. (2012). The sensitivity of neutrophil CD64 expression as a biomarker of bacterial infections is low in critically ill patients. *Intensive Care Med.* **38**, 445-452.
- ICARDI M., ERICKSON Y., KILBORN S., STEWART B., GRIEF B., SCHARWEBER G. (2009). CD64 Index provides simple and predictive testing for detection and monitoring of sepsis and bacterial infection in hospital patients. *J. Clin. Microbiol.* **47**, 3914-3919.
- LYNEMA S., MARMER D., HALL E.S., MEINZEN-DERR J., KINGMA P.S. (2014). Neutrophil CD64 as a diagnostic marker of sepsis: impact on neonatal care. *Am. J. Perinatol.* Doi: 10.1055/s-0034-1384644.
- MANCARDI D.A., ALBANESI M., JÖNSSON F., IANNASCOLI B., VAN ROOIJEN N., KANG X., ENGLAND P., DAERON M., BRUHNS P. (2013). The high affinity human IgG receptor Fc-gamma (CD64) promotes IgG-mediated inflammation, anaphylaxis and antitumor immunotherapy. *Blood.* **121**, 1563-1573.
- ROGINA P., STUBLJAR D., OSREDKAR J., SKVARC M. (2014). Expression of CD64 on neutrophils (CD64 index): diagnostic accuracy of CD64 index to predict sepsis in critically ill patients is better than of procalcitonin C-reactive protein, research note. *Clin. Chem. Lab. Med.* Doi: 10.1515/ccim-2014-0814.
- SANQUIST M., WONG H.R. (2014). Biomarker of sepsis and their potential value in diagnosis, prognosis and treatment. *Exp. Rev. Clin. Immunol.* **21**, 1-8.
- TEPHEN T., HUHN M., MELMER G., TUR M.K., BARTH S. (2009). Fc-gamma receptor 1 (CD64), a target beyond cancer. *Curr. Pharm. Des.* **15**, 2712-2718.
- WONG L., HILL B.L., HUNSBERGER B.C., BAGWELL C.B., CURTIS A.D., DAVIS B.H. (2014). Automated analysis of flow cytometric data for measuring neutrophil CD64 expression using a multi-instrument compatible probability state model. *Cytometry B Clin. Cytom.* Doi: 1002/cyto.b.21217.