ISSN : 2350-0743

www.ijramr.com

International Journal of Recent Advances in Multidisciplinary Research
Vol. 05, Issue 01, pp.3353-3356, January, 2018

RESEARCH ARTICLE

LOW LYMPHOCYTE-TO-MONOCYTE RATIO IS ASSOCIATED WITH AN ENHANCED REGULATORY T LYMPHOCYTE FUNCTION IN METASTATIC CANCER PATIENTS

[1]Paolo Lissoni, [1,*]Giusy Messina, [1]Franco Rovelli, [2]Luigi Vigorè, [3]Arianna Lissoni and [4]Giuseppe Di Fede

[1]International Institute of Psychoneuroendocrinoimmunology, Milan, Italy
[2]Laboratory of Immunology, San Gerardo Hospital, Monza, Italy
[3]Division of Surgery, Manzoni Hospital, Lecco, Italy
[4]Institue of Biological Medicine, Milan, Italy

ARTICLE INFO

Article History:
Received 20th October, 2017
Received in revised form
15th November, 2017
Accepted 25th December, 2017
Published online 30th January, 2018

Keywords:
Biomarkers,
Immunoinflammatory Response,
Macrophage System,
T Helper lymphocytes,
regulatory T lymphocyte

ABSTRACT

Despite their different mechanism of action, the antitumor efficacy of overall anticancer therapies would depend not only on their antitumor cytotoxic activity, but also on the immune biological response of cancer patients, which is the result of interactions between immunostimulatory and immunosuppressive events. Today, it is known that the anticancer immunity is mainly depending on T helper (TH) (CD4+) and dendritic cells, whereas it inhibited by the macrophage system and regulatory T lymphocytes (T reg) (CD4+CD25+). Moreover the generation of T reg cells has been proven to be stimulated by macrophage-mediated chronic inflammatory status related to cancer progression. Therefore, it becomes to be clinically important to identify possible inexpensive biomarkers able to investigate and to define the immunoinflammatory status of cancer patients with the respect to more expensive analyses, such as the measurement of lymphocytes subset and cytokines blood concentration. Recent clinical studies have shown the common available inexpensive routinely laboratory analyses, such as lymphocytes-to-monocyte ratio (LMR) may reflect the immunoinflammatory status of cancer patients, since lymphocyte and monocyte count are respectively related to the anticancer immunity and to his macrophage mediated suppression. On this basis, a study was planned to establish which correlation may exist between LMR and lymphocyte subsets, namely T reg cells, in metastatic cancer patients. The study included 30 metastatic cancer patients, who were affected by the most common solid neoplasm. LMR and TH-TO-T reg ratio were considered to be normal when they were greater than 2.1 and greater than 10. Abnormally low values of LMR and TH-TO-T reg ratio were observed in 14/30 (47%) and in 16/30 (53%) patients, respectively. Patients with abnormally low values of TH-TO-T reg ratio showed significantly mean values of LMR than those with normal values. Moreover, a significantly positive correlation was observed between TH-TO-T reg ratio and LMR values. On the contrary, LMR was negatively correlated to T reg cell percentage. The results of this preliminary study would suggest that LMR may represent the best surrogate inexpensive biomarker with the respect to other most complex and expensive immune analyses to monitor the status of the immunoinflammatory biological response of cancer patients.

INTRODUCTION

From a clinical point of view, the recent advances in cancer immunotherapy with cytokines such as IL-2 (Whittington, 1993) and anti-immune checkpoint monoclonal antibodies (MAB) (Ashwell, 2008) require the identification of biological markers to monitor and predict immunotherapy-induced biological response of cancer patients. Moreover, potential biological markers would have to be identified not only at tumor histological features, but also in the blood of cancer patients. Themselves Unfortunately, the evaluation of the immune status, commonly require several complex and expensive clinical analyses, including the determination of cytokine blood concentrations and lymphocyte subpopulations, in particular T helper (TH) and regulatory T lymphocytes (T reg), which are the main lymphocytes responsible for the activation and the suppression of the anticancer immunity, respectively (Riesco, 1979; Grimm *et al.,* 1982; Zou, 2006). Therefore, it is clinically important to reach the possibility to evaluate the anticancer immune status of each single cancer patients, mainly in those undergoing cancer immunotherapy through the common laboratory routinely analyses, by identifying simple and less expensive biological parameters. Today it is known that the human anticancer immunity is the end result of two fundamental biological response, the anticancer immune reaction and the chronic inflammatory response, which is mainly mediated by the macrophage system and is associated with a suppression of the anticancer immunity (Grivennikov *et al.,* 2010). The activation of an

***Corresponding author:* Giusy Messina,**
International Institute of Psychoneuroendocrinoimmunology, Milan, Italy.

effective anticancer immune response in humans is mainly a TH cell and dendritic cell phenomenon, whereas its suppression is namely mediated by T reg lymphocytes (Riesco, 1979; Grimm *et al.,* 1982; Zou, 2006). Then, the simple TH cell to T reg cell ratio has been proven to be a biological parameter able to synthetically reflect the interaction between activation and suppression of the anticancer immunity (Brivio *et al.,* 2008), which however cannot be commonly used in the clinical management of cancer patients because of its relatively high cost. The chronic inflammatory response allows a suppression of the anticancer immunity by either stimulating the generation of T reg cells (Thornton, 2002) or determining macrophage tumor infiltration (Mantovani *et al.,* 2008), which has been proven to stimulate cancer cell proliferation and to predict a poor prognosis (Peggs *et al.,* 2009). On the same way, the evidence of an increase in t reg cell percentage has appeared to be associated with a poor prognosis in metastatic cancer patients (Keir *et al.,* 2008). T reg cell-induced suppression of the anticancer immunity has been proven to be mediated by the activation of specific immune proteins expressed of cell surface of lymphocytes, the so-called immune checkpoints, the most important of them are represented by cytotoxic T lymphocyte antigen-4 (CTLA-4) (Grenader *et al.,* 2016) and programmed death-1 (PD-1) (Wu *et al.,* 2015), whose activation induces an inhibition of both TH cell and cytotoxic T lymphocyte functions.

Moreover, recent clinical studies have demonstrated that commonly routinely hemochromocytometric parameters, namely the lymphocyte-to-monocyte ratio (LMR), may play a prognostic significance by reflecting the anticancer immune and chronic imflammatory response occurring in the single cancer patient (Eo *et al.,* 2016). In more detail, since the activation of the anticancer immunity or of its suppression is respectively related to lymphocyte and to monocyte-macrophage system (Riesco, 1979; Grimm *et al.,* 1982; Zou, 2006), LMR would synthetically express the relation existing between activation and chronic inflammatory status-related suppression of the anticancer immune reaction, by potentially representing the most simple and economic laboratory parameter to monitor the anticancer immune status of patients. In addition, the simple monocyte count has been proven to reflect the general status of the monocyte-macrophage system responsible for T reg cell system activation and to correlate with the presence of tumor-associated macrophages and tumor macrophage infiltration (Ehrke *et al.,* 1982), which may stimulate tumor growth and determine a poor prognosis (Peggs *et al.,* 2009). Then, it has to be determined which relation may exist between LMR and T reg cell activation in cancer patients, and if there may be a correlation between those two laboratory parameters, routinely and economic the former and specific and expensive the latter. The present preliminary study was performed in an attempt to investigate the relation between LMR and lymphocyte subset percentage, namely that of TH and T reg cells, because of their fundamental role in the activation and in the suppression of the anticancer immunobiological response, respectively (Riesco, 1979; Grimm *et al.,* 1982; Zou, 2006).

MATERIALS AND METHODS

The study included 30 consecutive metastatic solid tumor cancer patients (M/F: 10/20, median age: 56 years, range 41-83). Eligibility criteria were, as follows: histologically proven metastatic solid tumor, measurable lesions, no chronic concomitant treatment with opioids or corticosteroids because of their immunosuppressive effects, and no previous chemotherapy for at least 3 month prior to study. Patients follows: breast cancer: 11; lung cancer 5 (squamous cell: 2, adenocarcinoma: 2, small cell: 1); pancreas adenocarcinoma: 5; colorectal cancer: 3; gastric cancer: 3; melanoma: 2; hepatocarcinoma: 1. dominant metastasis sites were, as follows: soft tissues: 10; bone: 1; lung: 3; liver: 9, liver plus lung: 1, brain: 3, peritoneum: 3. for the immune evaluations, venous blood samples were collected in the morning after an overnight fast. In each blood sample, we have measured lymphocyte count, monocyte number and some lymphocyte subpopulations, including TH lymphocytes (CD4), cytotoxic T lymphocytes (CD8), T reg (CD4+CD25+), NK cells (CD16+CD56+). Lymphocyte subsets were detected by the flow cytometric analysis and specific MABs, which were supplied by Becton7Dickinson (Milan, Italy). Moreover, LMR and TH-to-T reg ratio were also determined. According to the data reported in the literature (Riesco, 1979; Zou, 2006; Brivio *et al.,* 2008) and observed in our laboratory, lymphocyte count, LMR and TH-to-T reg ratio were considered to be within the normal range (95% confidence limits) when they were respectively more than 1,500/mm3, more than 2.1 and more than 10, corresponding to a percentage of T reg cells less than 10% with respect to that of TH lymphocytes. Data were reported as mean +/- SE, and statistically analyzed but the chi-quare test, the Student's test, and the coefficient of correlation, as appropriate.

RESULTS

Abnormally low lymphocyte count less than 1,500/mm3 was observed in 19/30 (63%) metastatic cancer patients. Moreover, abnormally low values of LMR less than 2.1 and TH-to-T reg ratio less than 10% were seen in 14/30 (47%) and in 16/30 (53%), respectively. Cancer patients with abnormally low values of TH-to-T reg ratio showed significantly lower mean values of LMR than those with TH-to-T reg ratio within the normal range (1.7 +/- 0.2 vs 4.4 +/- 0.6, $p<0.005$). LMR values in relation to the other immune parameters are reported in Table I. As shown, cancer patients with LMR higher than 2.1 showed statistically significantly higher mean number of lymphocytes ($p<0.05$), total T lymphocytes ($p<0.025$) and CD4+ lymphocytes ($p<005$) than those with low LMR value. On the contrary, patients with normal LMR had significantly lower mean count of monocytes ($p<0.025$) and T reg cell percentage ($p<0.001$) with respect to the values observed in patients with low LMR mean values, whereas no significant difference in T reg cell mean count was observed between patients with normal or abnormally low LMR. CD8+ lymphocyte and NK cell mean numbers were respectively higher and lower in patients with LMR values higher than 2.1 than in those with values less than 2.1, but none of these differences was statistically significant. Moreover, LMR was positively correlated to TH-to-T reg ratio ($r=0.79$, $p<0.025$). Table 2 shows T reg mean number, T reg percentage of TH cells and TH-to-T reg mean ratio in relation to lymphocyte count. Lymphocytopenia was associated with a statistically significantly lower TH-to-T reg ratio ($p<0.05$) and with a significantly higher t reg cell mean percentage ($p<0.05$) with respect to the values found in patients with normal lymphocyte count. Finally, the percentage of lymphocytopenia was significantly higher in patients with abnormally low LMR than in those with normal values (12/14 (86%) vs 7/16 (44%), $p<0.025$) and in patients with low TH-to-T reg ratio than in those with normal values (13/16 (81%) vs 6/14 (43%), $p<0.05$).

Table 1. Immune parameters (x +/- SE) observed in 30 metastatic cancer patients with normal (>2.1) or low lymphocyte-to-monocyte ratio (LMR)

IMMUNE PARAMETERS	LOW LMR (n=14)	NORMAL LMR (n=16)
LYMPHOCYTES (n/mm3)	1,030 +/- 89	1,724 +/- 219*
MONOCYTES (n/mm3)	760 +/- 127**	364 +/- 56
T LYMPHOCYTES (CD3) (n/mm3)	653 +/- 78	1,273 +/- 182**
T HELPER LYMPHOCYTES (CD4) (n/mm3)	364 +/- 56	762 +/- 127*
T CYTOTOXIC LYMPHOCYTES (CD8) (n/mm3)	254 +/- 32	415 +/- 61
REGULATORY T LYMPHOCYTES (CD4+CD25+)		
- n (mm3)	55 +/- 12	52 +/- 9
- CD4 percentage	13,2 +/- 1,1***	7,1 +/- 0,6
NK CELLS (CD16+CD56+) (n/mm3)	236 +/- 44	193 +/- 41
T HELPER-to-REGULATORY T LYMPHOCYTES RATIO	8,1 +/- 0,05	15,8 +/- 1,7 ***

* P < 0,05; ** P < 0,025; *** P < 0,001

Table 2. Regulatory T lymphocytes (T reg) mean percentage of T helper (TH) lymphocytes and TH-to-T reg mean ratio (TH/T reg) in metastatic cancer patients with normal lymphocyte count (> 1,500/mm3) and in those with lymphocytopenia

LYMPHOCYTES	n	T reg %	TH/T reg
LYMPHOCYTHOPENIA	19	10,5 +/- 0,6	10,2 +/- 0,6
NO LYMPHOCYTOPHENIA	11	7,3 +/- 0,6*	15,6 +/- 1,5*

* P<0,05

DISCUSSION

The results of this preliminary study, carried out to identify possible inexpensive biological markers able to synthetize the immunoinflammatory status of metastatic cancer patients in an appropriate manner similar to that of other more sophisticate, but more expensive biomarkers, such as cytokine blood levels and lymphocyte subsets, namely TH-to-T reg ratio (Riesco, 1979; Grimm *et al.,* 1982; Zou, 2006; Grivennikov, 2010; Brivio *et al.,* 2008), would suggest that the simple LMR evaluable on the basis of the simple hemochromocytometric analysis, is negatively correlated with the chronic inflammatory-immunosuppressive status of patients with metastatic neoplastic disease. Therefore, the evidence of an abnormally low LMR would reflect an exaggerated activation of macrophage-T reg lymphocyte system. Then, the same clinical information on the immunoinflammatory status of cancer patients, which may be drawn from sophisticate and expensive immune analyses, may be also obtained from the simple hemochrome by detecting MR, which could be considered a simple inexpensive surrogate biomarker, capable of showing the opposite relation between lymphocyte-induced anticancer immunity and its suppression by the macrophage-mediated chronic inflammatory status. Moreover, according to previous clinical investigations (Riesco, 1979; Grimm *et al.,* 1982; Zou, 2006; Grivennikov, 2010; Brivio *et al.,* 2008; Thornton, 2002), this study confirms that cancer-related lymphocytopenia is associated with an increased percentage of T reg cells with respect to that of TH lymphocytes.

The lack of significant difference in T reg cell mean count between patients with normal or low number of lymphocytes, which represent the main cells involved in the generation of an effective anticancer immunity (Riesco, 1979; Grimm *et al.,* 1982; Zou, 2006), but also on its association with an abnormally high percentage of T reg cells within the group of CD4+ cells. Obviously, the great variety of tumor histotypes considered in the present study does not allow us to identify possible immune difference in relation to the different tumor histotypes. If successive studies in a greater number of patients will confirm the clinical significance of LMR as a biomarker capable of synthetizing the interactions between anticancer immunity and its suppression by the chronic inflammatory status, LMR could be identified as a the most simple biological marker to investigate the immunoinflammatory status of cancer patients, namely during the metastatic disease, either in basal conditions or under the different anticancer conventional treatment, including chemotherapy itself, because of its potential stimulatory effect on the anticancer immune reaction.

REFERENCES

Whittington R, Faulds D. 1993. Interleukin-2. drugs 46: 446-483.

Ashwell S, Janetka JW, Zabludoff S. 2008. Keeping checkpoint kinases in line: new selective inhibitors in clinical trials. Exp Opin Invest Grugs 17: 1331-1340.

Riesco A. 1979. Five-years cancer cure: relation to total amount of peripheral lymphocytes and neutrophils. Cancer 25: 135-140.

Grimm EA, Mazumder A, Zhag HZ, Rosenberg SA. 1982. Lymphokine-activated killer cell phenomenon. *J Exp Med*., 155: 1823-1841.

Zou W. 2006. Regulatory T cells, tumor immunity and immunotherapy. Nat Rev Immunol 6: 295-307.

Grivennikov SI, Greter FR, Karin M. 2010. Immunity, inflammation and cancer. Cell 295: 883-899.

Brivio F, Fumagalli L, Parolini D, Messina G, Rovelli F, Rescaldini R, Vigorè L, Vezzo R, Vaghi M, Di Bella S. 2008. T-helper/T-regulatory lymphocyte ratio as a new immmunobiological index to quantify the anticancer immune status in cancer patients. In Vivo 22: 647-650.

Thornton F, Shevach EM. 2002. Suppressor effector function of CD4+CD25+ immunoregulatory T cells is a non-specific antigen. J Immunol 164: 183-190.

Mantovani A, Allavena P, Sica A, Balkwill F. 2008. Cancer-related inflammation. Nature 454: 436-444.

Peggs KS, Quezada SA, Chmbers CA, Korman AJ, Allison JP. Blockade of CTLA-4 on both effector and regulatory T cell compartments contributes to the antitumor activity of ant-CTLA-4 antibodies. J Exp Med 206: 1717-1725, 2009.

Keir ME, Butte MJ, freeman GJ, Sharpe A. PD-1 and its ligands in tolerance and immunity. Annu Rev Immunol 26: 677-704, 2008.

Grenader T, Waddell T, Peckitt C, Oates J, Starling N, Cunningham D, Briggerwater J. Prognostic value of neutrophil-to-lymphocyte ratio in advanced oesophago-gastric cancer: exploratory analysis of the REAL-2 trial. Ann Oncol 27: 687-692, 2016.

Wu G, Yao Y, Bai C, Zeing JU, Shi D, Gu X, Song Y. Combination of platelet-to-lymphocyte ratio and neutrophil-to-lymphocyte ratio is a useful prognostic factor in advanced non-small cell lung cancer patients. Thorac Cancer 6: 275-287, 2015.

Eo WK, Chang HJ, Kwon SH, Koh SB, Kim YO, Ji Yi, Kim HB, Lee JY, Suh DS, Kim KH, Chang IJ, Kim HJ, Chang SC. 2016. The lymphocyte-to-monocyte ratio predicts patients survival and aggressiveness of ovarian cancer. J Cancer 7: 289-296.

Ehrke MJ, Mihich E, Berd D, Mastrangelo MJ. 1982. Effects of anticancer drugs on the immune system in humans. Semin Oncol 16:230-239.

****