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FOCUSSED RESEARCH REVIEW

Immune biomarkers for chronic inflammation related complications in non-cancerous and cancerous diseases

Yaron Meirow[1] · **Michal Baniyash**[1]

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Abstract Chronic inflammation arising in a diverse range of non-cancerous and cancerous diseases, dysregulates immunity and exposes patients to a variety of complications. These include immunosuppression, tissue damage, cardiovascular diseases and more. In cancer, chronic inflammation and related immunosuppression can directly support tumor growth and dramatically reduce the efficacies of traditional treatments, as well as novel immune-based therapies, which require a functional immune system. Nowadays, none of the immune biomarkers, regularly used by clinicians can sense a developing chronic inflammation, thus complications can only be detected upon their appearance. This review focuses on the necessity for such immune status biomarkers, which could predict complications prior to their appearance. Herein we bring examples for the use of cellular and molecular biomarkers in diagnosis, prognosis and follow-up of patients suffering from various cancers, for prediction of response to immune-based anti-cancer therapy and for prediction of cardiovascular disease in type 2 diabetes patients. Monitoring such biomarkers is expected to have a major clinical impact in addition to unraveling of the entangled complexity underlying dysregulated immunity in chronic inflammation. Thus, newly discovered biomarkers and those that are under investigation are projected to open a new era towards combating the silent damage induced by chronic inflammation.

Keywords Immune biomarkers · Chronic inflammation · Immunosuppression · Cancer · Type 2 diabetes · MDSC

Abbreviations

5FU	5-fluorouracyl
A1c	Glycated hemoglobin
BM	Bone marrow
CAR-T	Chimeric antigen receptor T cell
CRC	Colorectal cancer
CTLA-4	Cytotoxic T lymphocyte associated protein 4
CVD	Cardiovascular disease
DC	Dendritic cell
HMGB1	High mobility group box 1 protein
hs-CRP	High sensitivity C-reactive protein
IBD	Inflammatory bowel disease
IFN-γ	Interferon gamma
ITAM	Immunoreceptor tyrosine-based activation motif
LDH	Lactate dehydrogenase
MDSC	Myeloid derived suppressor cells
MFI	Mean fluorescence intensity
Mo-MDSC	Monocytic MDSC
NO	Nitric oxide
PD1	Programmed death receptor 1
PD-L1	Programmed death receptor ligand 1
PMN-MDSC	Poly-morpho-nuclear MDSC

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✉ Michal Baniyash
michalb@ekmd.huji.ac.il

[1] The Lautenberg Center for General and Tumor Immunology, Faculty of Medicine, Israel-Canada Medical Research Institute, The Hebrew University, POB 12272, 91120 Jerusalem, Israel

RAGE	Receptor for advanced glycation end products
ROS	Reactive oxygen species
sICAM-1	Soluble intracellular adhesion molecule 1
SNX9	Sorting nexin 9
T2DM	Type 2 diabetes mellitus
TCR	T-cell receptor
TGF-β	Transforming growth factor beta
TIL	Tumor infiltrating lymphocyte
TNF-α	Tumor necrosis factor alpha
Treg	Regulatory T-cell
VEGF	Vascular-endothelial growth factor

Introduction

Dysregulated immunity under chronic inflammatory conditions, complications and clinical relevance

Chronic inflammation is becoming a major health care problem as it is considered "A Secret Killer" due to its "quiet" development, with no clinical signs, until complications are evident. Chronic inflammation develops when the immune system is unable to clear a persistent insult. This includes cases of immune evading pathogens, cancer, autoimmune diseases and chronic metabolic disorders [1, 2]. Chronic inflammation is characterized by continual recruitment of innate and adaptive immune cells, which produce high levels of pro-inflammatory molecules. This generates a harmful environment, resulting in tissue damage and increased risk of cancer initiation and progression. High levels of circulating pro-inflammatory molecules activate regulatory arms of the immune system, mainly myeloid derived suppressor cells (MDSCs), aiming to prevent an excessive immune activation. These lead to a dysregulated immunity and immunosuppression, weakening the immune effector arm and exposing the host to a myriad of complications [1]. These include increased susceptibility to opportunistic infections, tissue transformation, malignancies and other inflammatory insults. In such cases, infiltrating immune cells and factors together with prolonged secretion of activating signaling compounds lead to an unresolved inflammatory process that is reaching distant organs and thereby affecting healthy tissues.

Examples are evident in the association between chronic inflammatory bowel disease (IBD) and the increased risk of colon carcinoma, human papillomavirus and Hepatitis B and C virus with cervical and hepatocellular carcinoma, respectively, or *Helicobacter pylori* infection associated with gastric cancer [3–5]. Chronic inflammatory diseases can eventually affect every part of the body including internal organ systems and connective tissues, posing life-threatening conditions. Besides the increased risk of cancer, additional disease-related complications are found in association with developing chronic inflammation in non-cancerous pathologies, as in type 2 diabetes mellitus (T2DM). Chronic inflammation observed in diabetic patients is one of the leading causes of the disease-associated complications manifested by decreased kidney function, eye maladies, heart attacks and strokes [6]. Under all conditions, chronic inflammation leads to a disturbed homeostasis, tilting the physiological and immunological conditions towards a pro-inflammatory harmful environment involving cells and secreted factors. As chronic inflammation is recognized for being the major cause and a key player in complications evident in different types of diseases, its diagnosis and treatment constitutes an enormous challenge for the medical world.

The clinical need

Based on the sudden appearance of the wide range of complications, there is a tremendous need for biomarkers that can sense the inflammatory status, its effect on the immune system and predict complications before their occurrence. *In cancer*, the chronic inflammation and ensuing immunosuppression perpetuate the harmful tumor micro- and macro-environment, su pporting its growth and spreading, while preventing the success of given immune and chemo-based therapies. Hence, it should be mandatory to use biomarkers that sense the change in immune status generated by chronic inflammatory conditions and thus, predict success rates and follow the efficacies of such therapies. Moreover, some recently discovered biomarkers could be utilized for innovative intervention, targeting chronic inflammation itself. *In non-cancerous diseases*, as in diabetes, there is an urgent need to predict complications before they are evident, in order to treat the patients ahead of time and prevent disease deterioration. In all cases, contributing to patients' quality of life and saving health care expenses are the current dominant medical goals. At present, there are virtually no markers in use capable of segregating acute and chronic inflammation. Most commonly used inflammatory serum markers (such as PGE_2, high sensitivity C-reactive protein (hs-CRP), IFNγ, TNFα, IL-1β and a myriad of other soluble factors) are already up-regulated in the acute phase, and therefore unsuitable for detecting the development of chronic inflammation. The next section describes the use of cellular biomarkers in peripheral blood samples for sensing a developing chronic inflammation by following the suppressive and the suppressed immune cell milieus.

Immune system biomarkers that sense chronic inflammation and the ensuing immunosuppression

Monitoring the affecting cells

MDSCs

A massive amount of data has accumulated in the past 30 years regarding accumulation and polarization of suppressive myeloid cells which were later termed MDSCs, in various diseases characterized by chronic inflammation, posing them as established responders and mediators of chronic inflammation. Thus, monitoring MDSCs in patients may present a more coherent picture of their inflammatory profile and the ensuing immune status.

MDSCs are a heterogeneous population of immature myeloid cells sharing a common immunosuppressive activity. MDSCs can be identified by flow-cytometry as $Gr1^{+}CD11b^{+}$ cells in mice. These can be further subdivided into $Ly6C^{hi}Ly6G^{-}CD11b^{+}$ monocytic MDSCs (Mo-MDSCs) and $Ly6C^{lo}Ly6G^{+}CD11b^{+}$ poly-morphonuclear MDSCs (PMN-MDSCs). Human MDSCs are generally characterized as $HLA\text{-}DR^{lo/-}CD33^{+}CD11b^{+}$. Human Mo-MDSCs are $HLA\text{-}DR^{lo}CD33^{+}CD11b^{+}CD14^{+}CD15^{-}$ and PMN-MDSCs are $HLA\text{-}DR^{-}CD33^{+}CD11b^{+}CD14^{-}CD15^{+}$ [7]. Under normal conditions MDSCs are retained in the bone marrow (BM) and migrate to the periphery where they differentiate into DCs, macrophages and neutrophils, while losing their suppressive activity. High levels of pro-inflammatory growth factors (GM-CSF and VEGF [8]), chemokines (CXCL10 [9], CXCL12 [10], CCL5 [11] and CCL2 [8]), and cytokines (TNF-α, IFN-γ, IL-1β, IL-6 and TGF-β [12]) in chronic inflammation lead to the polarization of MDSCs. Polarized MDSCs accumulate in the BM and the periphery in their immature form while increasing their suppressive activity and causing a general immunosuppression. Based on their phenotype, MDSC numbers could be evaluated by flow cytometry analyses and serve as biomarkers indicating chronic inflammation-induced immunosuppression, as described by numerous studies analyzing blood samples from cancer patients. The potential use of MDSCs as biomarkers in non-cancerous inflammatory diseases was previously reported in studies on diseases such as systemic lupus erythematosus [13], autoimmune arthritis [14], IBD [15] and in HIV 1 infection [16]. However, measurable levels of MDSCs combined with additional molecular MDSC biomarkers, which reflect their immunosuppressive features/activity, could strengthen the certainty of the evaluation of the patients' immune status.

Molecular features of MDSCs as biomarkers

Several molecular features in MDSCs, correlating with their suppressive activity, have been previously shown to increase in human diseases. These could assist in determining MDSC activity and the overall severity of chronic inflammation and related immunosuppression.

Production of *nitric oxide* (*NO*) and *reactive oxygen species* (*ROS*) by MDSCs is related to their suppressive activity [12, 17]. Little is known about the mechanism by which NO and ROS suppress T-cell activity. However, it has been shown that nitration and nitrozylation of T-cell surface molecules by MDSCs result in an aberrant T-cell receptor (TCR) complex assembly and reduced T-cell function [18]. Intracellular staining of NO and ROS and detection by flow cytometry in whole blood samples shows their increased production by MDSCs in colorectal cancer (CRC) [19], melanoma [12, 20] and non-small-cell-lung-carcinoma [21] in addition to their increased levels.

PD-L1 (*CD274*) is the ligand for the inhibitory receptor on T-cells, programmed-death-receptor-1 (PD1). PD-L1 is expressed on both Mo-MDSCs and PMN-MDSCs and may be involved in their suppressive activity through PD-L1/PD1 interaction. PD-L1 expression on Mo-MDSCs has been shown to increase along the progression of melanoma, peaking at stage III and stage IV [9]. Similar analyses show an increased expression of PD-L1 on PMN-MDSCs in metastatic CRC patients [22]. This cell surface marker can be detected by flow-cytometry.

The pro-inflammatory calcium binding *S100A8/A9 proteins* are produced by MDSCs. The expression of these proteins is greatly increased in MDSCs during chronic inflammation, along with the expression of their receptor; receptor for advanced glycation end products (RAGE) [12]. S100A8/A9 induce MDSC differentiation arrest, maintaining them in their suppressive state. Serum levels of S100A8/A9 proteins measured by ELISA have been shown to increase in melanoma patients, representing a poor prognosis [20]. S100A9 can also be detected by intracellular staining with commercially available monoclonal antibodies, applicable for flow-cytometry.

CD39 and CD73, which work together in conversion of extracellular ATP into adenosine, have been shown to be involved in T-cell suppression [23, 24]. Recently, Limagne et al. have found these two receptors are expressed on PMN-MDSCs and to a lesser extent on Mo-MDSCs, as detected by flow cytometry. The expression of CD39 and CD73 was augmented on MDSCs isolated from blood of metastatic CRC patients. Furthermore, blocking the activity of these enzymes restored T-cell function in vitro, showing their direct involvement in the suppressive activity of these MDSCs [22].

Regulatory T-cells as biomarkers for chronic inflammation

The use of regulatory T-cells (Tregs) as biomarkers for chronic inflammation and immunosuppression is advantageous, as it not only follows the amount of Tregs directly, but also demonstrates MDSC activity indirectly due to their ability to induce Tregs in vivo [25, 26]. Several recent studies have followed peripheral blood Tregs in human diseases; Treg frequency has been shown to increase along the progression of melanoma, peaking at stage III-IV [9]. In CRC patients, lower frequency of circulating Tregs corresponded to a better prognosis [23]. Although there are discrepancies as to the prognostic meaning of tumor infiltrating Tregs, which have been described to provide a good prognosis in certain reports or a bad one in others [27, 28], circulating Tregs seem to reflect the general immune status. They are increased in chronic inflammation and their numbers correlate with disease severity.

Monitoring the affected cells

One of the most prominent effects of MDSCs in chronic inflammation is T- and NK-cell dysfunction. Direct assessment of the severity of the immunosuppression and T-cell dysfunction in chronic inflammation is only possible by ex vivo activation of the T-cells and assessment of their proliferation and cytokine production. This method is time and resource consuming and clinically impractical. Finding molecular markers in the dysfunctional T-cells, which mirror MDSC levels and their suppressive activity, may provide the complementary data needed to fully assess patients' immune status.

CD247—the T-cell receptor ζ chain

MDSCs have been shown to inhibit proliferation, cytokine production and killing ability of T- and NK-cells both in vitro and in vivo [12, 29–31]. This dysfunction is associated with down-regulation CD247, the TCR ζ chain. CD247 is normally expressed in all peripheral T- and NK-cells and is associated with the TCR and the NK killing receptors NKp46, NKp30 and FcγRIII (CD16). It is a 16 kDa trans-membrane protein, which contains three immunoreceptor tyrosine-based activation motifs (ITAMs), making it the central signaling subunit of its associated receptors. Studies performed in mouse models have shown that the presence of MDSCs during chronic inflammation induces lysosomal degradation of CD247 without affecting the surface expression of the remaining TCR subunits, leaving functionally cryptic and inactive complexes [31]. CD247 down-regulation is a reversible phenomenon, as the resolution of the chronic inflammation and/or elimination of MDSCs results in restoration of normal CD247 expression and T-cell functions [29]. Thus, CD247 expression levels in T- and NK-cells are sensitive to changes in MDSC suppressive activity and can be easily quantified by flow-cytometry analyses. Indeed, CD247 down-regulation has been shown in blood samples collected from patients suffering from various diseases of different etiologies, compared to healthy donors. Moreover, most studies have found an inverse correlation between the down-regulation of CD247 and the level of MDSCs in patients with various cancer types [19, 32–35], chronic hepatitis C [36], HIV infection [37] and type II diabetes mellitus [38].

Sorting nexin 9—a novel biomarker for MDSC induced suppression

In the course of our studies, we came across a manuscript published in 2002 by Schaefer et al. [39], which described a sequence within the human immunodeficiency virus type 2 Nef protein, found to interact with CD247 and lead to its internalization. We hypothesized that an endogenous protein, similar to the Nef protein might be expressed in T-cells during chronic inflammation and lead to CD247 down-regulation. A protein blast of the Nef protein sequence that binds CD247 in the human proteome data resulted in a single human protein, which contains an identical sequence, and was identified as sorting nexin 9 (SNX9). Our study quickly focused on examining a possible interaction between SNX9 and CD247 and its involvement in CD247 down-regulation during chronic inflammation. SNX9 is a member of a family of sorting nexins, also including SNX18 and SNX33, described to be involved in many basic cellular processes such as endosomal sorting, clathrin mediated endocytosis, cell division and invadopodia formation [40, 41]. SNX9 is ubiquitously expressed in most cell types including immune and cancer cells. Ish-shalom et al. [32] have indeed found an association between SNX9 and CD247 in T-cells, however no evidence could be found to its involvement in CD247 down-regulation. Surprisingly, under chronic inflammatory conditions SNX9 was dramatically downregulated in mouse T- and B-cells. This finding was repetitive in various models of diseases characterized by chronic inflammation. Moreover, like CD247, SNX9 down-regulation was mediated directly by MDSCs both in vitro in co-culture of T-cells and MDSCs, and in vivo as SNX9 down-regulation has been reversed by MDSC depletion. In addition, fewer MDSCs were required to induce SNX9 down-regulation than the amount needed for the induction of CD247 down-regulation both in vitro and in vivo, suggesting that SNX9

is more sensitive to the presence of MDSCs. Moreover, while SNX9 was more sensitive to the induced chronic inflammation and associated immunosuppression than the CD247, the latter was more sensitive to the recovery stage upon MDSC neutralization; CD247 expression levels recovered faster than SNX9. The development of a new monoclonal antibody enabled the detection of SNX9 in human peripheral blood leukocytes by flow-cytometry [32]. Using the new method, Ish-shalom et al. [32] have detected such down-regulation also in T-cells in blood samples collected from advanced CRC patients, compared to healthy donors. Thus, measurement of SNX9 expression in blood samples can sensitively detect the increasing MDSC activity in the development of chronic inflammation and provide an earlier warning of impending immunosuppression, while CD247 is a more sensitive biomarker for the immune system recovery, upon MDSC and chronic inflammation neutralization.

Monitoring strategies

The biomarkers described above and new ones to be discovered are expected to form a solid basis for the development of an immune system biomarker algorithm for the evaluation of the patient's immune status with high sensitivity and accuracy. Levels of cellular biomarkers as MDSCs and Tregs are currently determined by flow cytometry analysis of blood samples, the limiting factor being sample handling and analysis; fresh versus frozen whole or Ficoll separated blood samples. Determining percentage is relatively accurate if compared to the right controls. Measuring changes in expression levels of affected intracellular molecules and compounds (CD247, SNX9, S100A8, S100A9, NO and ROS) is currently performed by flow cytometry and presented by the mean fluorescent intensity (MFI) in a particular cell population. However, depending on the molecule of interest the sensitivity and accuracy of the tests display high variability. ELISA assays are more complicated to be used when assessing a whole cell population, as the specific cells of interest must be first isolated/sorted prior to measurement of the indicated molecule(s). The isolation procedures remove the cells from the original environment, which could lead to changes in the cell features and thus, to inaccurate conclusions. Circulating molecules such as cytokines and chemokines could be detected in the patients' sera by ELISA assays, which are sensitive and accurate when using the appropriate controls. Today, a combination of the above described biomarkers could allow diagnosis, providing a "yes" or "no" resolution most likely only for the patients positioned in the extreme groups. Unfortunately, there are still intermediate patients with certain levels of immunosuppressive biomarkers that their diagnosis is inconclusive. Therefore, identifying more biomarkers could help performing a better diagnosis and prognosis for this group of patients. Organ pathology, tumor and clinical parameters will have to be considered in all cases.

The clinical use of the newly described immune system biomarkers

In cancer

Immune cells can kill cancer cells but on the other hand, can provide supportive conditions for tumor growth and invasion under pro-inflammatory conditions developing during chronic inflammation. The popularity of taking the immune/inflammatory milieu into account in cancer prognosis and follow-up is rising in recent years when cancer treatments are designed according to the following concerns: (1) The inflammatory milieu has been described to directly support tumor growth and spreading. (2) Perpetuated chronic inflammation during tumor growth leads to immunosuppression. (3) The generated immunosuppressive environment affects not only the patient's immune status but also the efficacy of a variety of immune and chemo-based anti-cancer therapies. Indeed, cumulative clinical data point at moderate success rates of ~20–40%, depending on the type of tumor, of such immune-based therapies. These are costly and may cause side effects, not falling from those of chemotherapy. Thus, there is an urgent need for new immune biomarkers to define responder versus non-responder patients, to choose the former for immediate treatment and offer the latter alternative combined treatments to recuperate their immune system and create an optimal supportive environment to the novel immune-based therapies.

MDSCs and CD247 as diagnostic biomarkers in disease progression and follow-up of anti-cancer treatments

The clinical use of immune biomarkers that reflect the patients' systemic immune status provide new insights into the design of anti-cancer treatment strategies, as the traditional classification systems of cancer could not fulfill this need. Accumulating evidence suggest that MDSCs and their accompanied immunosuppressive milieu could serve as markers for poor prognosis and disease progression in different types of cancer. We had recently reported that in stage-IV melanoma patients, HLA-DR$^{-/low}$CD33^{+}CD11b^{+} MDSC levels increase in the peripheral blood alongside disease progression and metastases classification [33]. Additional publications show an increase in peripheral HLA-DR$^{-/lo}$CD14^{+} Mo-MDSCs in the progression of breast cancer [42], multiple myeloma [43], pancreatic

adenocarcinoma [44], CRC [22] and melanoma [9]. In some of the cases the combination of MDSC levels, suppressive features and CD247 expression were used. In such cases, level of circulating MDSCs could provide invaluable insight as to which patients are at an increased risk of disease progression, health deterioration and higher mortality [9].

As changes in the disease staging and accordingly, in the ensuing inflammatory micro- and macro-environments are evident following various anti-cancer therapies, such immune biomarkers could be potentially used as sensors to follow-up the effects of conventional anti-cancer treatments as chemotherapy, radiotherapy and surgery.

Chemotherapy is meant to directly target and kill the dividing cancer cells. However, accumulating evidence highlight its effect on the immune system. Therefore, innovative ways could be used to measure chemotherapy efficacies while taking advantage of the immune biomarkers that sense changes in the immune system, which correlate with the disease stage. Sevko et al. have tried to explain the limited efficiency of low dose cyclophosphamide treatment, commonly used to treat melanoma due to its tendency to induce immunogenic cell death and inhibit Tregs. It was established that the low dose cyclophosphamide treatment in mice with spontaneous melanoma resulted in the accumulation of $Gr1^{+}CD11b^{+}$ suppressive MDSCs. Moreover, treatment of inflamed mice with low dose cyclophosphamide resulted in an increased production of NO and ROS by MDSCs, which was associated with increased suppressive activity. Only these new findings could explain the inefficiency of the promising treatment with low dose cyclophosphamide [45]. Kanterman et al. have previously shown adverse effects of certain chemotherapies on MDSCs and the related immunosuppression using colitis-induced CRC in mice. Treatment with CPT11 increased levels of $Gr1^{+}CD11b^{+}$ MDSC levels and ROS production, along with immunosuppression associated with low CD247 expression in T-cells and increased mortality rate compared to untreated tumor bearing mice. On the other hand, 5-fluorouracyl (5FU) had beneficial effects; diminishing MDSC levels along with their production of NO and ROS, alleviating immunosuppression as evident by a normal expression of CD247, decreased tumor load and increased survival, compared to untreated tumor bearing mice [19]. These results were further corroborated in stage IV CRC patients. The study followed two cohorts of patients: one treated with FOLFIRI, which contains CPT11 together with 5FU, and another with FOLFOX, which contains oxaliplatin in combination with 5FU [19]. Patients who were treated with FOLFIRI had higher levels of $HLA\text{-}DR^{-}CD33^{+}CD11b^{+}$ circulating MDSCs and low CD247 expression, while patients who were treated with FOLFOX had fewer circulating MDSCs and higher CD247 expression. These results were in concurrence with the clinical outcome of the treatment as the patients who were treated with FOLFOX survived longer. Limagne et al. have followed patients who suffer from metastatic CRC and were treated with FOLFOX in combination with bevacizumab (Avastin) for 12 months. Their findings showed increased probability of survival in patients who had low PMN-MDSCs and Mo-MDSCs before getting the treatment. Moreover, while following the patients along the course of treatment, a decrease in PMN-MDSC frequency provided a much better prognosis and significantly higher probability of survival than those who had an increase in PMN-MDSC frequency [22]. In these three examples the authors show the importance of following the immune based biomarkers, namely MDSCs, during treatment. It becomes clear that the immune status is critical in achieving a successful and efficient chemotherapy and must be followed.

Radiofrequency ablation is a commonly used procedure to treat non-small-cell-lung-carcinoma. However, in some cases the procedure fails to eliminate all the tumors, resulting in recurrence of the disease. Schnider et al. have followed patients with recurrent tumors and compared them to patients who underwent complete ablation. Patients who had an incomplete ablation showed an early increase in serum levels of MDSC related factors like TNF-α, CCL-2 and CCL-4. Although the ablation did not affect MDSC frequency in those patients, NO production in MDSCs after radiotherapy was also increased at an early stage, indicative of an incomplete ablation. The authors suggested that these markers can be used as early sensors for the efficiency of radiofrequency ablation [21].

Surgical resection of tumors is the main treatment option for CRC patients. However, limited data has been shown regarding its effect on the immune status, MDSCs and chronic inflammation. OuYang et al. have followed such patients, measuring the frequencies of MDSCs, Tregs, $CD8^{+}$ T-cells and NK-cells in their peripheral blood before and one week after the surgery. Although no change was observed in the frequencies of the effector cells ($CD8^{+}$ T-cells and NK-cells), tumor resection led to a significant reduction in $HLA\text{-}DR^{-/lo}CD33^{+}CD11b^{+}$ MDSCs and Tregs. This suggests that surgically decreased tumor load reduces chronic inflammation in the macro-environment, favoring effector cell activity rather than immunosuppression [23]. Thus, following the suppressive immune status could also determine the efficacy of tumor resection, giving early signs of complications or disease remission.

MDSCs as prognostic markers for follow-up of immune based anti-cancer therapy efficacy and prediction of responsiveness

In recent years, the utilization of immune-based modalities in treatment of cancer has been on the rise. These treatments aim to boost the endogenous anti-tumor immune responses or apply effector cells to combat the tumor when the endogenous immune system is unable to do so. Anti-cancer vaccines such as Sipuleucel-T in prostate cancer, melanoma peptide/tumor vaccines and personalized lymphoma vaccines, are based on presentation of tumor antigens by DCs to enhance endogenous T-cell cytotoxic activity towards tumor eradication, a process requiring a functional patient's immune system [46]. Another strategy is to use tumor-infiltrating lymphocytes (TILs) that are extracted from tumors, activated and expanded ex vivo, then returned to the patients with the hope that the enhanced activity of the tumor specific T-cell clones will combat the tumor. Chimeric antigen receptors T (CAR-T) cell treatments involve adoptively transferred CD8 T-cells expressing engineered anti-tumor receptors and are expected to kill the tumor cells [47]. Another class of anti-cancer immune based therapies is immune checkpoint blockers such as anti-CTLA-4 and anti-PD1, which target inhibitory checkpoint molecules with the purpose of prolonging T-cell cytotoxic activity [48]. The prerequisite for the success of all the above-mentioned treatments is a permissive environment and functional immunity. Therefore, chronic inflammation poses a major obstacle in immune-based therapeutic strategies. Several groups have reported difficulties in the ex vivo generation of mature DCs (required for establishment of cancer vaccines) when the prevalence of $CD14^+HLA^-DR^{-/low}$ MDSCs was high in the initial samples taken from patients with glioblastoma, prostate cancer and non-Hodgkin lymphoma [49–52]. Moreover, the presence of MDSCs hinders the activity of cancer vaccine as shown for small-cell–lung-carcinoma and renal cell carcinoma [53, 54]. TIL treatment in a mouse melanoma model, has also been shown to be inefficient when MDSCs are present. Blocking of CSF-1R in this model resulted in decreased numbers of MDSCs and restored TIL cytotoxic activity [55]. MDSCs have also been shown to inhibit the killing ability of engineered chimeric antigen receptor T-cells in mice [56]. This effect was reversed by reduction of the number of MDSCs with all-*trans* retinoic acid [57]. Several studies have correlated the overall survival of advanced melanoma patients treated with ipilimumab (anti-CTLA-4), to the level of MDSCs in their circulation. Patients who have elevated numbers of MDSCs were less likely to respond to the treatment and had significantly shorter survival [58, 59]. Moreover, the level of $HLA\text{-}DR^-CD33^+CD11b^+$ MDSCs in blood samples of melanoma patients provided more accurate prediction of the response to ipilimumab treatment than the classically used lactate dehydrogenase (LDH) measurements [33]. It was also shown that a high baseline frequency of MDSCs, $Lin^-HLA^-DR^{lo/-}CD14^+CD11b^+CD33^+$ Mo-MDSCs and high levels of IL-6 are associated with a reduced chance of responding to ipilimumab treatment [60]. Others proposed that increased eosinophils and absolute lymphocyte count, along with low LDH, MDSCs, Tregs and related inflammatory factors as S100A8/A9 and HMGB1, can be used as novel, complex predictive markers for patients who may benefit from the ipilimumab therapy [20, 61].

Hence, screening for immune based markers associated with chronic inflammation in blood samples taken from candidates for immunotherapy, may serves as a coherent strategy for determining which patients would benefit from such treatments. A routine follow-up of such biomarkers during treatment and thereafter could identify patients in high risk of disease recurrence.

In Diabetes

Type 2 diabetes mellitus (*T2DM*) is a complex metabolic disorder associated with an increased risk of micro- and macro-vascular diseases; its main clinical characteristic is hyperglycaemia [62]. Low grade chronic inflammation is underlying the pathogenesis associated with T2DM. High levels of circulating free lipids, as observed in obesity, lead to adipocyte hypertrophy, activation of resident macrophages and release of pro-inflammatory mediators, resulting in insulin resistance and related complications [6, 63]. Although much progress has been made during the years in the identification of risk factors associated with T2DM, its healthcare and socioeconomic impact is increasing, mainly because of its associated complications [64]. T2DM approximately doubles the risk of a wide range of cardiovascular diseases (CVD) and is also associated with a wide range of non-vascular diseases, including cancer, mental and nervous system disorders, infections and liver disease [65].

One of the foremost medical problems related to T2DM patients is the inability to predict the related complications prior to their diagnosis, as well as the lack of available parameters for measuring competency of given therapies. Currently, the diagnosis of complications in T2DM patients is done upon their appearance. Although the association between low grade chronic inflammation and T2DM is widely recognized, the issue of causality and the degree to which inflammation contributes and serves as a risk factor for the development of disease-associated complications

remain unresolved. A major part of this uncertainty is due to a general lack of sensitive and specific biomarkers of low-grade chronic inflammation or alternatively of its consequences [66].

Various biomarkers of inflammation as hs-CRP, TNF-α, lipoprotein (a), and sICAM-1, have been associated with disease progression and their levels were found to be significantly increased in diabetic patients with existing coronary artery diseases [67–69], nephropathy [2, 68, 70, 71], and retinopathy. However, these parameters could not serve as biomarkers that predict complications nor indicative of chronic inflammation or the patients' immune status. As previously reported, conflicting results exist as to the correlation between hs-CRP and diabetes-related micro- and macro-vascular complications and its relevance as a biomarker of disease progression [72]. Moreover, as hs-CRP levels do not distinguish between acute and chronic inflammation and are not indicative of responses to certain anti-inflammatory medications such as statins and aspirin [69], its frequent measurements in diabetic patients might have a limited usefulness in the clinic.

A non-inflammatory biomarker that is currently being used for the diagnosis and prognosis of diabetes is the glycated hemoglobin (A1c). Although this biomarker has many advantages, the fact that it represents average results of a relatively long period, which is affected for example by the change in diet and various treatments or related diseases such as anemia, makes it less relevant for periodic examination of the patient's condition. Arguably, it reveals little as to the actual pathologic inflammatory process underlying the progression of micro- and macro-vascular complications [73, 74].

In light of these studies, more process-specific biomarkers of the pathologic inflammation in diabetes are needed. Presently, most biomarkers used for evaluation of diabetes severity and progression, rely on metabolic parameters and are limited in improving risk stratification regarding disease-related outcomes [74]. Identifying pertinent biomarkers that are linked to the metabolic processes as well as the underlying inflammatory process could have a major clinical impact on the prediction of disease progression and efficacy of therapeutic interventions.

A biomarker that may shed light on the level of chronic inflammation and/or its consequence is therefore expected to both improve the understanding of the factors contributing to the progression of complications in diabetes and offer a tool for assessing therapeutic efficacy and novel therapeutic targets. Our results showing that chronic inflammation leads to the generation of MDSCs, which induce a bystander T-cell immunosuppression associated with CD247 down-regulation, were described in a variety of chronic inflammatory diseases [1, 75]. Consequently, we hypothesized that the CD247 expression levels in T cells that serve as a biomarker sensing chronic inflammation and associated immunosuppression could be associated with parameters of disease severity/progression in T2DM patients and consequently, predict diabetes related complications. Indeed, our studies revealed that CD247 expression levels in T cells could serve as a sensitive and predictive biomarker for CVD and nephropathy in diabetic patients, possibly linking glycemic disease processes with the inflammatory process [38]. Our study, although confounded by its relatively small cohort size and short follow-up time, presented promising results that depicted a novel approach to risk stratification in diabetes.

It is hereby suggested that measurements of CD247 levels in T cells could shed new light on the actual pathologic process leading to organ damage, rather than following only the typical markers of the diabetic metabolic disorder such as glycemia and lipids. Therefore, understanding the underlying immunological processes during diabetes development and progression could highlight novel treatment modalities which will combine therapies directed at the metabolic disturbances and at the resultant chronic inflammatory processes towards optimizing personalized treatments of T2DM patients.

Concluding remarks

Taken together, the cumulative data summarized in this review highlights the critical role of immune networking in non-cancerous and cancerous diseases characterized by chronic inflammation and their associated complications. The susceptibility of patients to developing chronic inflammation is a result of cumulative harmful effects and is defined by an imbalanced homeostasis. Importantly, many therapeutic treatments employed up to date fail to produce a desirable effect, since a permissive immune environment is a prerequisite for their proper function. Nowadays, there is no doubt that chronic inflammation and associated immunosuppression pose a serious obstacle in the prognostic and the therapeutic area, as they both develop with no palpable clinical signs, often leading to unforeseeable complications and possible unresponsiveness to various therapies.

The biomarkers for immune monitoring described in this review are presented as 'stand-alone' measurements, which correlate with disease severity and response to treatment. The ultimate goal is to generate an immune status scoring system based on a combinatorial use of such biomarkers. More data must be gathered before such a system could be established, as so far there are no consensus standards to any of these measurements. This will allow stratification or dichotomization of patients according to single and multiple markers, determining their chance of responding to treatment and following treatment efficacy. Patients

Table 1 Examples for the use of immunological biomarkers for monitoring the immune status in diseases characterized by chronic inflammation

Disease	Biomarkers	Corresponding to	Refs.
Melanoma	%$CD14^+CD11b^+HLA\text{-}DR^{lo}$ Mo-MDSCs (high), IL-1β, IFN-γ and CXCL10 (increased serum levels), PD-L1 expression on Mo-MDSCs (increased expression)	Disease progression	[9]
	%$HLA\text{-}DR^{-/lo}CD33^+CD11b^+$ MDSCs (high), NO and ROS (increased production by all MDSCs)	Disease progression, advanced metastases and poor response to ipilimumab treatment	[33]
	%$HLA\text{-}DR^{-/lo}CD14^+CD11b^+$ $CD33^+$ Mo-MDSCs (high)	Poor response to ipilimumab treatment	[20, 58, 60]
Colorectal cancer	%$HLA\text{-}DR^{-/lo}CD33^+CD11b^+$ MDSCs (increase along treatment), CD247 (low expression in T-cells), NO and ROS (increased production by all MDSCs)	Beneficial effect of FOLFOX, compared to FOLFIRI regimen	[19]
	%$HLA\text{-}DR^{-/lo}CD14^+$ Mo-MDSCs (increase) %$CD14^+CD33^+HLA\text{-}DR^{lo}$ Mo-MDSCs and %$CD15^+CD33^+HLA\text{-}DR^-$ PMN-MDSCs (high before treatment)	Disease progression Poor response to FOLFOX-bevacizumab treatment	[22]
	%$HLA\text{-}DR^{-/lo}CD33^+CD11b^+$ MDSCs (decrease after resection)	Efficacy of surgical resection	[23]
Non-small cell lung carcinoma	TNF-α, CCL2, CCL4 (increased serum levels), NO (increased production by Mo-MDSCs)	Efficacy of radiofrequency tumor ablation	[21]
Breast cancer	%$HLA\text{-}DR^{-/lo}CD14^+$ Mo-MDSCs (increase)	Disease progression	[42]
Multiple myeloma	%$HLA\text{-}DR^{-/lo}CD14^+$ Mo-MDSCs (increase)	Disease progression	[43]
Pancreatic adenocarcinoma	%$HLA\text{-}DR^{-/lo}CD33^+$ MDSCs (increase)	Disease progression and poor ECOG score	[44]
Glioblastoma, prostate cancer and non-Hodgkin lymphoma	%$HLA\text{-}DR^{-/lo}CD14^+$ Mo-MDSCs (high in PBMC samples extracted from patients)	Inefficient ex vivo maturation of DCs hinders production of DC based vaccines	[49–51]
Small cell lung carcinoma and renal cell carcinoma	%$HLA\text{-}DR^{-/lo}CD33^+$ MDSCs (high before treatment)	Poor response to autologous DC based vaccine	[53, 54]
Type II diabetes mellitus	CD247 (low expression in T-cells)	Disease progression and severity, prediction of cardiovascular events and nephropathy	[38]
Systemic lupus erythematosus	%$HLA\text{-}DR^-CD11b^+CD33^+$ MDSCs (increase)	Active disease	[13]
Rheumatoid arthritis	%$CD33^+CD11b^+$ MDSCs (increase)	Active disease	[14]
Inflammatory bowel disease	%$HLA\text{-}DR^{-/lo}CD14^+$ Mo-MDSCs (increase)	Disease exacerbation	[15]
HIV 1 infection	%$HLA\text{-}DR^{-/lo}CD33^+CD14^+CD15^-$ Mo-MDSCs (increase along with viral load)	Disease severity	[16]

categorized as possible poor responders can then be offered treatments to alleviate the chronic inflammation to increase their chance of response. Currently, there is already a limited array of immune biomarkers available to clinicians who should start using them in the routine check-ups and follow-ups of patients suffering from chronic diseases.

The fact that some of the immune biomarkers are suppressive cells such as MDSCs and Tregs poses them as targets for ablation, which could relieve the harms of chronic inflammation, associated immunosuppression and direct support of cancer, to increase treatments' efficacies. In addition, follow-up of patients for the biomarkers' levels will enable the evaluation of the disease progression and sense disease recurrence. In non-cancerous conditions, such as in T2DM, a follow-up of changes in the immune system biomarkers, combined with the convectional glycemic measurements, is expected to have a significant impact on the possibility to predict complications' appearance that could be treated a head of time towards a better quality of life and the prevention of disease deterioration. The examples presented in this review are summarized in Table 1.

Thus, although monitoring the recently described immune biomarkers is expected to serve as a powerful tool in improving the quality of life of patients suffering from chronic inflammatory diseases, the discovery of additional new biomarkers is necessary to validate and increase the certainty of conclusions, regarding the clinical management of diseases and the design of new therapeutic strategies.

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Compliance with ethical standards

Conflict of interest No conflicts of interest were disclosed.

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