

## **Role of Biomarkers in Risk, Diagnosis, Response to Treatment, and Prognosis of the Autoimmune Diseases**

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# CONTENTS

## Chapters:

**1-Pathophysiology of Multiple Sclerosis**

**2- Role of Biomarkers in Risk Assessment, Diagnosis, Response to Treatment, and Prognosis of Multiple Sclerosis**

**3- Pathophysiology of Oral Lichen Planus**

**4- Role of Biomarkers in Risk Assessment, Diagnosis, Response to Treatment, and Prognosis of Lichen Planus**

**5- Pathophysiology of Psoriasis**

**6- Role of Biomarkers in Risk Assessment, Diagnosis, Response to Treatment, and Prognosis of Psoriasis**

**7- Pathophysiology of Rheumatoid Arthritis**

**8- Role of Biomarkers in Risk Assessment, Diagnosis, Response to Treatment, and Prognosis of Rheumatoid Arthritis**

**9- Pathophysiology of Crohn's Disease**

**10- Role of Biomarkers in Risk Assessment, Diagnosis, Response to Treatment, and Prognosis of Crohn's Disease**

**11- Role of Biomarkers in Risk Assessment, Diagnosis, Response to Treatment, and Prognosis of Other Autoimmune Diseases**

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ROLE OF BIOMARKERS IN RISK, DIAGNOSIS, RESPONSE TO T...

## 1-PATHOPHYSIOLOGY OF MULTIPLE SCLEROSIS

Given to the controversies with respect to the pathophysiology of progressive multiple sclerosis (MS), we address both aspects of inflammation and degeneration in mediating progression. In addition to treatments that target the inflammatory and degenerative aspects, we also discuss treatments that improve remyelination, given that reformed myelin may restore trophic support to denuded axons to reduce axonal degeneration. Although the focus of this chapter is on progressive MS, we also refer to significant relevant findings from other inflammatory and neurodegenerative disorders, such as neuromyelitis optica spectrum disorder, given to similarities in their pathological processes. As the study populations assessed in different trials differed significantly regarding their inclusion criteria and changed over time, comparison of the findings of different investigations is difficult.

### *Neuroinflammation*

RRMS is known by immune cell-driven, plaque-like demyelination with consequent neurodegeneration, whereas the invasion of T cells into the CNS parenchyma is considerably lower

albeit still present in progressive MS. Furthermore, in progressive MS, inflammation is thought to be compartmentalized at both the leptomeningeal and the blood vessel levels within the CNS parenchyma, as few breaches are thought to exist in the blood brain barrier. Infiltration of monocytes which differentiate into macrophages and local stimulation of microglia is observed in all types of MS, but the macrophage/microglia representation is more significant in progressive MS, and these cells are thought to drive the expansion of established plaques. The normal-appearing white matter (NAWM) contains diffuse microglial inflammation with few hypercellular plaques and inflammatory perivascular cuffs. Moreover, oxidative injury (including to mitochondria), which is considerably contributed to by macrophages/microglia, is common in progressive MS plaques. Given that progressive MS is known by a mostly intact blood–brain barrier, treatments for progressive MS that target pathophysiological characteristics of progressive MS should cross the blood–brain barrier, have the ability to inhibit the function of microglia, decrease oxidative stress and retain the ability to change the function of T lymphocytes and B lymphocytes.

### **T Lymphocytes**

The aim of therapeutic modulation of T cell composition and differentiation in progressive MS is

commonly to normalize inflammation and reduce any involvement of T cell infiltrates. T cells can be identified in the spinal cord parenchyma of cases with PPMS, but, unlike those of cases with RRMS, these T cells are identified at lower levels and are more diffuse in the NAWM and normal-appearing grey matter (NAGM). Interestingly, despite the low level of T cell-mediated inflammation in progressive MS plaques, levels of inflammatory mediators of T cell activation in cerebrospinal fluid (CSF) are comparable between cases with progressive MS and those with RRMS. In contrast to these low levels of T cells in the parenchyma of cases with progressive MS, enhanced numbers of T cells are located within the meninges in cases with progressive MS. The extent of meningeal T cell inflammation is associated with axonal loss in the NAWM, proposing that the detrimental role of T cells in progressive MS is potentially carried out by long-range soluble factors. In progressive MS types, T cells slowly increase in spaces connecting the leptomeninges to the parenchyma, such as Virchow–Robin spaces and meninges. This result may suggest that progressive MS is not known by a lack of T cell activation, but rather by exclusion of T cells from the brain and spinal cord parenchyma. Moreover, tissue-resident memory T cells, such as the few that are identified in the CNS parenchyma of cases with progressive MS, tend to have downregulated expression of S1PRs in comparison with central memory or effector memory T cells, which is probably why these cells

are retained in tissues and not changed by S1PR modulators such as fingolimod or siponimod.

### **B Lymphocytes**

B cells play multiple roles and contribute to progression in MS34 via antibody synthesis, antigen presentation to T cells and the production of pro-inflammatory cytokines. Investigations of brains from subjects who died with progressive MS have shown increased cellular inflammatory aggregates, specially in the meninges, some of which are enriched with B cells and have been referred to as ectopic follicles. The significance of this meningeal B cell accumulation is shown by findings of subpial grey matter demyelination and microglial function in cortical structures. Meningeal B cell accumulation is thought to be clinically relevant as cases with B cell follicular structures have a faster progression of disability, an earlier disease initiation and a younger age at death than those without such structures. In progressive MS, the existence of a subset of B cells (DC-SIGN+ B cells) and T helper cells (ICOS+ TFH cells) associates with disease progression, proposing the presence of ongoing peripheral inflammation. Moreover, clonally expanded plasma cells from cases with MS synthesize antibodies directed against neurons and astrocytes, but rarely against myelin; however, these antibodies result in demyelination in murine spinal cord slice cultures, stressing the pathological role

of clonally expanding B cells in progressive MS. On the contrary, new evidence reveals that the number of IgA+ plasma cells is decreased in the gut during EAE, and that over-abundance of IgA+ plasmablasts or plasma cells results in resistance against EAE development. These findings are similar to the results of a clinical investigation in cases with RRMS, in which weekly treatment with atacicept a soluble fusion protein containing a fragment of transmembrane activator and CAML interactor (TACI; a TNF receptor superfamily member) that binds to B lymphocyte stimulator (BLYS; also known as BAFF) and a proliferation-inducing ligand (APRIL) resulted in deterioration with a higher annualized relapse rate in the atacicept groups than in the placebo group. These findings reveal that the role and type of plasma cells or different lineage precursor stages of B cells in different organs can be either beneficial or deleterious, speaking to the complexities of potential therapeutic interventions. Therefore, B cell depletion has been known as a promising intervention to the management of progressive MS and is under current clinical evaluation.





## **2- ROLE OF BIOMARKERS IN RISK ASSESSMENT, DIAGNOSIS, RESPONSE TO TREATMENT, AND PROGNOSIS OF MULTIPLE SCLEROSIS**

### ***Biomarkers of Axonal Damage***

#### ***Neurofilament Light Chain***

Over the past three decades, many methods have been developed to evaluate neurofilament light chain (NfL) concentrations. Neurofilaments are cytoskeletal proteins secreted from damaged axons into the CSF and the blood. Investigations have also shown that increased cNfL levels are associated with increased CD4<sup>+</sup> T lymphocytes, which have been implicated in the inflammation reported in MS, and progression of RRMS to SPMS. Early investigations have shown that cNfL levels of MS cases are increased during active relapse and acute relapse compared to healthy subjects. There is a positive association between cNfL and serum NfL (sNfL) in MS cases, with cNfL levels 42-fold higher than sNfL levels. A benefit to using sNfL levels as opposed to cNfL levels is that serum levels are easier to evaluate than performing a spinal tap or lumbar puncture on a case to retrieve CSF. Over the last

few years, single molecular array (SiMoA) has made evaluating NfL concentration levels more clinically relevant.

Generally, MS cases also had higher sNfL levels before treatment compared to healthy subjects. With disease-modifying treatment, sNfL levels were lower. Managements with higher efficacies have also been revealed to decrease NfL levels more effectively than traditional therapeutic interventions. Moreover, levels of sNfL have been correlated with T2 lesion volumes. Some investigations revealed strong associations between sNfL levels and the number of active plaques present on MRI scans. However, some cases have several active MRI plaques with low sNfL, and some have no MRI plaques with high sNfL, suggesting that other confounding factors can lead to high sNfL levels. Therefore, cases will still need MRI scans. Investigations have also revealed that brain and spinal cord atrophy may be positively associated with sNfL levels. One investigation revealed a decrease in the brain and spinal cord volume over five years, with a greater decrease for those with higher sNfL baseline concentrations.

### **Amyloid-Precursor Protein**

Amyloid precursor protein (APP) has also been found in Alzheimer's disease but nevertheless may be correlated with MS. It is synthesized by astrocyte cells during demyelination and can be found in

reactive glial cells during de- and remyelination. MS cases present with higher concentrations of APP compared to healthy subjects and axons that are positive for APP in MS cases have been found to be associated with CNS plaque development.

### **Tubulin Beta**

It has been characterized that Tubulin *beta* (TUB $\beta$ ) is a subunit of tubulins, which are heterodimeric proteins that make up microtubules. The neuron development and regeneration have been correlated with increased synthesis of the class II tubulin isotype. Specifically, one investigation showed that CSF TUB $\beta$  was increased in cases with MS compared to cases with other neurological disorders.

### **Biomarkers of Neuronal Injury**

#### **14-3-3 Protein**

14-3-3 protein, which is present in neurons, can be assessed in the CSF of both cases with MS and those with Creutzfeldt-Jakob disease. However, the role of 14-3-3 protein in MS is conflicting. Investigations have shown that 14-3-3 protein in the CSF is correlated with more severe disability, more extensive involvement of the spinal cord, and faster progression to MS or disease progression. Early accumulation of 14-3-3 protein in the CSF may be associated with reduced rates of recovery. However, some investigations have difficulties

detecting 14-3-3 protein in the CSF, with one only showing it in 2 cases out of 22 MS cases and another showing it in only one case out of 21 CIS cases.

### ***Neuron Specific Enolase***

Levels of neuron specific enolase (NSE), an enzyme detected in neurons and axons that can be applied to estimate neuronal density, have been shown to be increased in both the CSF and serum of cases suffering from trauma, hypoxic brain injury, or cerebral bleeding. One investigation showed a decrease in serum and CSF NSE in cases with CIS compared to healthy subjects, with some investigations indicating either no change or a negative association between plasma NSE levels and EDSS and Multiple Sclerosis Severity Score (MSSS).

### ***Biomarkers of Glial Dysfunction***

#### ***Glial Fibrillary Acidic Protein***

Glial fibrillary acidic protein (GFAP) is produced by mature astrocytes and has been shown to be increased in plaques of MS cases, showing injury to astrocytes. Cases with SPMS had higher CSF GFAP levels than those with RRMS. Moreover, higher CSF levels of GFAP are correlated with greater disabilities and relapse.

#### ***S100 $\beta$ Protein***

There have been results of an increase in serum

and plasma concentrations of S100 $\beta$  protein, a subunit of the S100 protein that is detected in glial cells, in MS, with the highest levels in cases with PPMS or SPMS. Examples of S100 $\beta$  activities include maintaining astrocyte integrity, assisting with neuronal proliferation, and differentiating oligodendrocytes. During acute exacerbations in cases with RRMS, S100 $\beta$  levels have also been revealed to be increased; however, the window is small as S100 $\beta$  levels were no longer increased in cases who had acute exacerbations prior to one week ago. Changes in S100 $\beta$  have also been found in cases with cerebral ischemia of amyotrophic lateral sclerosis. However, an investigation did not show a statistically significant difference in CSF and serum S100 $\beta$  protein level between CIS cases and healthy subjects, but this may be confounded by some samples being received more than one week after the acute exacerbation. The same investigation also did not show any significant association between S100 $\beta$  protein levels and EDSS score. No differences in plasma S100 $\beta$  protein levels between various clinical subtypes of MS have also been reported, along with no difference in CSF S100 $\beta$  levels between cases with MS and controls.

#### ***Anti-Aquaporin 4 Antibodies***

Astrocytes express aquaporin-4 (AQP4) to assist establish homeostasis in the CNS by moving water through cell membranes. However, investigations

have shown that AQP4 is undetectable in cases with MS. This is a intervention to help with the difficult task of differentiating MS from neuromyelitis optica (NMO), which is a rare condition that also found with demyelination of the optic nerve and spinal cord.

### ***Biomarkers of Myelin Biology/Demyelination***

#### ***Myelin Basic Protein***

Myelin basic protein (MBP) is synthesized by the oligodendrocytes from the central nervous system and has been shown to be increased in the CSF of cases with MS and associated with EDSS scores. One investigation showed that MS cases with an acute exacerbation had higher concentrations than those with slower progressive MS and even higher than those in remission. However, using MBP is challenging, as demyelination plaques can be remyelinated by MBPs in the CSF.

#### ***Myelin Oligodendrocyte Glycoprotein***

Myelin oligodendrocyte glycoprotein (MOG) correlated disease, a newly recognized disease, is differentiated from NMO and MS by the presence of MOG antibodies in the serum. Moreover, differences have been shown in CSF myeloid cell types in cases with neuroinflammation, which included those

with MS and anti-MOG disease.

### ***Biomarkers of Immunomodulation and Inflammation***

#### ***Immune Mediators and Cytokines***

Pro-inflammatory cells, T helper (Th) 1 and Th17 cells, synthesize cytokines, such as interleukin (IL)-17, interferon (IFN)- $\gamma$ , and tumor necrosis factor (TNF)- $\alpha$ , while anti-inflammatory cells, regulatory T (Treg) and Th2 cells, synthesize IL-10 and IL-4. Evaluating these cytokines and cellular changes can indicate the disease type, confirmed by an investigation in pediatric MS where serum concentrations of anti-inflammatory cytokine IL-10 were predictive of relapse compared to other pro- and anti-inflammatory mediators. Therefore, immune signatures, along with the above markers all together as a composite, can be applied to more differentiate underlying disease pathology and disease severity.

Furthermore, C-X-C motif chemokine ligand (CXCL) 13 has been shown to be associated with worse prognosis and exacerbations in RRMS and conversion of CIS to MS. However, CXCL13 is non-specific as cases with infections also had high concentrations. One investigation reported that CSF and plasma levels of eotaxin-1 (CCL11) were

correlated with disease duration, especially in cases with SPMS. They also showed C-C motif chemokine ligand (CCL) 20 to be correlated with disease activity and CSF levels of IL-12B, macrophage inflammatory protein (MIP)-1a, cluster of differentiation (CD)5, and CXCL9, and plasma levels of oncostatin (OSM) and hepatocyte growth factor (HGF) to be correlated with MS. Serum IL-6 has also been shown to be associated with the age of onset for MS cases and was found at a higher rate in MS cases compared to healthy subjects.

Immune signatures may also predict treatment response or prognosis of MS cases. Most biomarker investigations have focused on IFN- $\beta$ , where there is a wide variation in response to this treatment. Neutralizing antibodies (Nabs) against IFN- $\beta$  are correlated with treatment failure, but they only partially clarify non-responsiveness. Serum cytokine levels have proposed immunologically distinct subgroups of MS, and these subgroups may stratify treatment response to IFN- $\beta$ .

### 3- PATHOPHYSIOLOGY OF ORAL LICHEN PLANUS

#### *Inflammatory Immune Response*

OLP commonly features a cytotoxic CD8+ T-cell epithelial infiltrate bordering apoptotic basal keratinocytes, overlying a helper CD4+ T-cell infiltrate of the lamina propria; however, the stimulating factor(s) and the pathogenic process of these immune responses are still unclear. In an investigation of the reactivity of lesional and nonlesional T-cell clones from LP cases against lesional and nonlesional autologous keratinocytes, the lesional T-cell lines in cases with LP were considerably more cytotoxic against autologous lesional keratinocytes than the T-cell line from clinically normal skin. Moreover, most cytotoxic clones from LP plaques were CD8+ and most noncytotoxic clones from LP plaques were CD4+. Taken together, the cytotoxicity of lesional CD8+ T cell can be inhibited by antimajor histocompatibility complex (MHC) class I monoclonal antibody.<sup>2</sup> These results propose that lesional cytotoxic CD8+ T cell may be activated by a basal keratinocyte antigen related to MHC class I, consequently promoting keratinocyte apoptosis. This eventual stimulating antigen has

not yet been found in OLP plaques. OLP plaques reveal an increased number of helper CD4+ T cell and Langerhans cells. Langerhans cells and keratinocytes can both express MHC class II in OLP plaques. In OLP plaques, therefore, helper CD4+ T cells may be activated by antigen related to MHC class II presented by Langerhans cells or keratinocytes. Interleukin-12 produced by MHC class II expressing lesional Langerhans cells and keratinocytes may promote a Thelper-1 CD4+ T-cell production of interleukin-2 and interferon- $\gamma$ . These T-helper-1 cytokines, together with the presentation of an antigen related to MHC class I on basal keratinocytes, trigger cytotoxic CD8+ T-cell induction of keratinocytes apoptosis.

### ***Correlation Between Viruses and OLP***

The viruses for which a correlation with OLP has been suspected can be divided into two groups: (1) viruses for which a correlation with OLP has been anecdotally proposed, and (2) viruses for which there is abundant documentation of a correlation with OLP, although with marked geographic disparities. In the first group can be included varicella zoster virus, Epstein-Barr virus, cytomegalovirus, human herpesvirus 6, human papillomavirus, and HIV. The second group essentially includes HCV. Along with porphyria cutanea tarda and cryoglobulinemia, OLP is one

of the three dermatologic disorders that have been most commonly reported in cases infected by the HCV. Since it was first reported in 1991 by a French team, the correlation between OLP and HCV-seropositivity has been well reported in Mediterranean populations, in Asia, and in the United States. Nevertheless, a correlation between OLP and HCV-seropositivity could not be approved in France, the United Kingdom, and in regions with high HCV prevalence, such as Egypt and Nigeria. A recent systematic review of HCV prevalence in LP cases and in matched controls without LP yielded 25 relevant reports, including eight with only OLP. A significantly higher rate of HCV-seropositivity was found in cases with OLP, with an odds ratio of 5.7. This correlation was stronger in Mediterranean regions and thought to disappear in Northern Europe. Conversely, in HCV-positive cases, the estimated prevalence of LP is 1.6% to 20%, being higher than expected in the respective geographic regions. The marked geographic heterogeneity of the correlation between OLP and HCV proposes that the stimulating of OLP in HCV-positive cases may need a peculiar genetic background (as noted earlier). Interestingly, Italian investigators showed that in HCV-positive cases, the occurrence of OLP is significantly correlated with the MHC class II allele DR6.

### ***The Stimulating Antigen:***



### ***A Self-antigen?***

The antigen that stimulates the inflammatory immune response in OLP plaques is yet unknown. This stimulation may be a self-antigen or an exogenous antigen. Several reports approve the intervention of an autoimmune response in OLP plaques: disease chronicity, adult onset, female prevalence, correlation with other autoimmune diseases, approval of autolytotoxic T cells in OLP plaques, development of OLP like plaques in cases with GVH disease, and effectiveness of immunosuppressor treatment.

### ***The Stimulating Antigen: Other Candidates***

When treatments or dental materials trigger plaques similar to OLP, the term “oral lichenoid reactions” is commonly used. Drug-induced oral lichenoid reactions have been mostly shown with nonsteroidal anti-inflammatory agents, angiotensin-converting enzyme inhibitors,  $\beta$ -blockers, methyldopa, and penicillamine. Many other treatments have been correlated with OLP, although with a low level of evidence. Several investigators also have shown that oral lichenoid reactions can result from contact hypersensitivity to dental materials such as amalgam, composite, and dental acrylics. Moreover, resolution of oral lichenoid reactions can also be found after replacement of the causative dental material.



#### **4- ROLE OF BIOMARKERS IN RISK ASSESSMENT, DIAGNOSIS, RESPONSE TO TREATMENT, AND PROGNOSIS OF LICHEN PLANUS**

##### ***Antioxidant and Peroxidation Level***

It has been reported that in cases with OLP the salivary level of peroxidation products such as malonaldehyde (MDA) and 8-hydroxydeoxyguanosine (8-OHdG) increased and that of antioxidants such as vitamin C and E were reduced compared with control healthy subjects. After therapy with curcumin, for more than two hundred days, the level of peroxidation products (oxidants) reduced and that of antioxidants increased. Therefore, level of vitamin C and E, as antioxidants, may be an appropriate biomarkers to predict precancerous conditions like OLP. Furthermore, investigations have shown the presence of serum anti-gastric parietal cell autoantibody (GPCA) in several different groups of OLP cases. Group of GPCA-positive OLP has signs and manifestations of oral lichen planus in forms of reticular, erosive or ulcerative oral mucosal plaques and pain or burning sensation of lesional

oral mucosa. Moreover, treatment of OLP cases with levamisole plus vitamin B12 revealed a significant reduction in GPCA level. This decrease in GPCA level was in line with improvement of buccal mucosa plaques. Consequently, GPCA level of serum is a potential biomarker to diagnose oral lesion planus.

### **Cortisol**

In an investigation, the salivary cortisol are shown to be associated with the incidence of OLP, and one may consider the cortisol levels of salivary as a potential marker for the development or creation of OLP plaques. In a study, it has been shown that the levels of salivary cortisol, dehydroepiandrosterone (DHEA), and psychological factors in cases with OLP. In that investigation, 31 cases with OLP were matched with same number of healthy subjects in terms of age and sex. The Beck test was applied to evaluate the level of anxiety and depression in the cases. They found no significant difference between salivary cortisol level in OLP group and control group. In another investigation, Ghalyani and Tavangar, (2010) showed lesser salivary cortisol level in cases with lichen planus than those non-infected subjects. Furthermore, it has been shown that cortisol level in cases with OLP was significantly lower than control subjects. Moreover, SCL-90 questionnaire findings revealed that the anxiety and depression was higher in these cases. Taken together, despite of some controversies in

association between cortisol and OLP, since cortisol results in a decrease in the number of lymphocytes and other immune cells, any dysfunction in the HPA (Hypothalamic– pituitary–adrenal axis) in cases with anxiety and depression results in decrease in blood and salivary cortisol production, and make diseases affecting the immune system like lichen planus.

### **Immunoglobulin**

Sistig et al., assessed cases with lichen planus and showed increased levels of IgG and IgA among them. Ghalayani et al., investigated the level of IgA and IgG in cases with OLP and lichenoid reaction plaques. The findings revealed higher level of IgA and IgG in cases than normal subjects in both groups. An increase in the salivary IgA level was found in cases with oral leukoplakia, OLP and carcinoma of the oral cavity. Furthermore, in another investigation IgA level in cases with OLP was higher than healthy subjects, although this difference was not significant.

### **Apoptosis-Related Biomarkers**

Changes of apoptosis mechanisms proved to be involved in general in the initiation of malignant cascades. Two main mechanisms of apoptosis have been reported, namely, the intrinsic (mitochondrial) and extrinsic (death receptor) mechanisms. Certain stimuli, such as hypoxia or free radicals, stimulate

the onset of the intrinsic mechanism inducing an increased permeability of mitochondrial pores, followed by the secretion of proapoptotic molecules, such as cytochrome c and apoptosis-inducing factor (AIF), from mitochondria into the cell cytoplasm. Cytochrome c stimulates procaspase 9, leading to formation of the apoptosome. This mechanism is carried out by B-cell lymphoma protein 2 (BCL-2) family and proteins including proapoptotic proteins (BCL-2-associated X protein (BAX), BCL-2 antagonist killer 1 (BAK), BCL-2 antagonist of cell death (BAD), BH3 interacting-domain death agonist (BID), etc.) and antiapoptotic proteins (BCL-2, BCL-2-related protein (BCL-XL), BCL-2-like 2 protein (BCL-W), myeloid cell leukemia-1 (MCL-1), etc.).

### **p53**

*p53* is the pivotal tumor suppressor gene located on chromosome 17 producing one of the main proteins, p53, involved in the prevention of carcinogenesis. This protein has role in DNA repair and destruction of defective cells through the stimulation of apoptosis. Therefore, mechanisms such as cell cycle arrest, apoptosis, and senescence are governed by the stimulation of p53.

Under normal conditions, p53 level is low as a consequence of rapid proteolysis: p53 is inactivated by mouse double minute 2 homolog (MDM2), which increases the degradation of p53 by proteasomes. In p53-induced apoptosis, mRNA increases for BID;

p53 also stimulates p53 upregulated modulator of apoptosis (PUMA) and NOXA expression, followed by the secretion of BAX and BAK from their complexes with antiapoptotic proteins and consequently mitochondrial outer membrane channel formation. Furthermore, p53 secretes BAK from the complex that the latter forms with MCL-1; therefore, BAK protein becomes available for mitochondrial pore formation. Another process by which p53 protein has role in the initiation and progress of apoptosis is the increase in transcription of p53-regulated apoptosis-inducing factor-1 (p53AIF-1) protein; p53AIF-1 is a protein that is identified in mitochondria, and its action involves the dissipation of the mitochondrial transmembrane potential, an important event of the intrinsic apoptotic mechanism followed by the cytosolic secretion of the cytochrome c and other mitochondrial proapoptotic proteins.

### **MDM2 and SUMO-1**

There is a strong association between p53, MDM2, and small ubiquitin-like modifier 1 (SUMO-1), molecules involved in cell proliferation and apoptosis. P53 is inactivated by MDM2 that enhances p53 proteasomal degradation. MDM2 acts as an E3 ubiquitin ligase for p53: following ubiquitination under the action of MDM2, p53 will be massively degraded by proteasomes, resulting in a reduction in p53 level and, consequently, to the

apoptosis inhibition. Moreover, it seems that MDM2 can restrain p53 activity by forming a complex with p53.

As for MDM2, its level is changed by SUMO-1; under normal conditions, MDM2 is undergoing self-ubiquitination and proteasomal degradation; in case of DNA injury, SUMO-1 binds MDM2 and abrogates its self-ubiquitination, resulting in an increase in MDM2 ubiquitin ligase function towards p53. In this particular manner, SUMO-1 regulates MDM2 level and, subsequently, p53 level.

Katayama et al. have shown that overexpression of MDM2 as an effect of SUMO-1 overexpression may act as a marker of tumor development and aggressiveness even in OSCC's early phases. In this regard, SUMO-1 in conjunction with MDM2 may be employed not only as an indicator for tumor occurrence but also as a possible target for future pharmacological interventions.

However, another investigation that has assessed the expression of proteins p53, MDM2, and SUMO-1 in 4 disorders localized on the oral mucosa compared to normal mucosa proved p53 and MDM2 overexpression in OLP, approving hence a promalignant transformation environment. As for the expression of SUMO-1 in OLP, it was shown to be similar in both normal mucosa and inflammatory fibrous hyperplasia, implying that changes of SUMO-1 develop at later phases of carcinogenesis, as an important overexpression of this protein was

shown in oral epithelial dysplasia and established OSCC.

## 5- PATHOPHYSIOLOGY OF PSORIASIS

### *Immunopathology of Psoriasis*

GPP is a rare, neutrophilic skin disease known by sudden episodes of widespread rash and sterile pustules that can occur with or without systemic inflammation, as denoted by fever, leukocytosis, and increased C-reactive protein levels. A key feature of GPP is repeated episodes of generalized sterile pustule formation resulted from extensive neutrophilic and mononuclear inflammatory infiltrates in the epidermis. Severe flares commonly need intensive hospital management.

GPP may present with pre-existing plaque psoriasis, and closely associated with immunologic mechanisms seem to underpin the pathogenesis of both conditions. However, GPP has also been revealed to present independently and is now known as a clinical entity separate from plaque psoriasis, with clear distinctions in genetic and immunologic determinants as well as in response to therapy. Understanding these distinctions is important to the development of effective, targeted treatments for this disease. GPP is predominantly known by innate immune inflammation and is noted an autoinflammatory

pustular neutrophilic disease. In this regard, GPP is known as representative of autoinflammatory keratinization disorders, which are known by inflammation in the epidermis, hyperkeratosis, and primary genetic causative factors correlated with the hyperactivation of innate immunity (autoinflammation). By contrast, plaque psoriasis has both innate and adaptive immunopathogenic reactions and is known as an autoimmune condition. Investigations have shown different cytokine mechanisms that are important in the presentation of each disease. For example, while the interleukin (IL)-23/IL-17 axis seems to drive plaque psoriasis, a growing body of evidence proposes that the IL-36 mechanism as central to the development of GPP.

IL-36 cytokines belong to the IL-1 family and are produced by and act upon different cell types, including keratinocytes, epithelial cells, and immune cells, in an autocrine or paracrine manner. With a pivotal role in modulating the innate immune system, the uncontrolled expression and activation of IL-36 cytokines can result in self-perpetuating inflammatory mechanisms. IL-36 signaling occurs by a complex of IL-36 receptor (IL-36R) and IL-1R accessory protein, propagating inflammatory processes in the epithelium. Dysregulation of this signaling mechanism seems to be central to the immunopathogenesis of GPP. Overexpression of IL-36 agonists (IL-36 $\alpha$ ,  $\beta$ , or  $\gamma$ )

or expression of a dysfunctional IL-36R antagonist (IL-36RA), produced by *IL36RN*, can result in a positive feedback loop of uncontrolled signaling and excess synthesis of inflammatory mediators. This in turn results in the stimulation of chemokines such as CXCL1 and CXCL8 (IL-8), synthesizing a chemokine gradient that attracts a high number of neutrophils into the epidermis.

More evidence on the differences in immunopathologic determinants driving GPP and plaque psoriasis pathogenesis comes from gene expression evaluations. Compared with healthy subjects, levels of tumor necrosis factor- $\alpha$ , IL-1, IL-17A, and IL-36 are elevated in skin biopsy samples from cases with GPP or plaque psoriasis; however, GPP plaques were proposed to have higher levels of IL-1 and IL-36 and lower levels of IL-17A and interferon- $\gamma$  in comparison with plaque psoriasis lesions. Immunohistochemical analysis also shown that IL-36 expression localizes to keratinocytes that surround the neutrophilic pustules in GPP. Moreover, GPP plaques contain higher levels of neutrophilic chemokines and neutrophil and monocyte transcripts in comparison with plaque psoriasis lesions. It is crucial to consider that IL-36 and IL-23 cytokine mechanisms closely interact and may crosstalk extensively, and dysregulation of either mechanism is capable of perpetuating an inflammatory process.

In addition to histologic evaluations, the key role

of the IL-36 mechanism in GPP is supported by the detection of related mutations in cases with the disease. To date, *IL36RN* mutations seem to be the main determinant of pathology in subjects whose disease has a known genetic component. These genetic mutations stimulate a response mechanism whereby IL-36R-activating ligands (IL-36 $\alpha$ ,  $\beta$ , and  $\gamma$ ) are not modulated by IL-36RA, resulting in self-amplifying IL-36 synthesis, notably in highly differentiated epidermal keratinocytes. *IL36RN* mutations resulting in an aberrant IL-36RA with reduced affinity for its receptor were found in an investigation of Tunisian families with a severe form of GPP known as deficiency of IL-36R antagonist (DITRA). In an international study, 7.7% of cases with GPP were heterozygous and 21% had biallelic mutations in *IL36RN*, whereas in an investigation of Chinese cases, *IL36RN* mutations were found in 75% of cases with GPP. Therefore, the occurrence of *IL36RN* mutations may differ by ethnicity, suggesting the likelihood of genetic diversity in the pathophysiology of GPP. GPP may often appear with existing or prior plaque psoriasis but can also arise in cases with no history of plaque psoriasis. Evidence that GPP alone is a distinct subtype of psoriasis, with its own etiology, comes from an investigation in Japanese cases in which *IL36RN* mutations were found in a much higher rate of cases with GPP alone compared with those presenting with

GPP and plaque psoriasis. *IL36RN* mutations are also correlated with an earlier age of onset and more severe GPP; moreover, the initiation of GPP has been shown to be substantially delayed in subjects with monoallelic compared with biallelic *IL36RN* mutations, proposing a gene dosage effect.



## **6- ROLE OF BIOMARKERS IN RISK ASSESSMENT, DIAGNOSIS, RESPONSE TO TREATMENT, AND PROGNOSIS OF PSORIASIS**

### ***Interleukin-6 (IL-6)***

IL-6 is one of the most crucial inflammatory mediators. It is produced by different cells such as: monocytes, fibroblasts, endothelial cells and adipocytes upon exposure to appropriate stimuli. Particularly interesting is the investigation performed by Fujishima et al. The investigators revealed that IL-17F can stimulate production of IL-6, both in normal human epidermal keratinocytes (NHEKs) and in mouse skin. It is worth noting that NHEKs produced much higher amounts of IL-6 after induction by IL-17F than by IL-17A or TNF-alpha. The investigators propose that IL-17F may be a pivotal cytokine that stimulates IL-6 expression in NHEKs. IL-6 acts as a chemotactic mediator for T cells, therefore it triggers the migration of T cells into the epidermis. IL-6 affects the growth and differentiation of dermal and epidermal cells and also has role in hematopoiesis. It has been shown that the production of IL-6 in fat tissue and its circulating levels are positively associated with obesity, inappropriate glucose



tolerance, and resistance to insulin. Furthermore, a decrease of body weight is correlated with decreased levels of this interleukin, as well as decrease of its production in fat tissue. Esteve et al. showed that serum IL-6 level was negatively associated with sensitivity to insulin, whereas it revealed a positive association with BMI, blood pressure values and triglyceride levels. It was also showed that IL-6 level was positively associated with intima media thickness. Nishida et al. proposed considering serum level of IL-6 as a marker of mechanisms which may result in early arterial changes in men. Significantly enhanced serum concentrations of IL-6 have been shown in psoriatic cases. A positive association between increased IL-6 levels and PASI has also been confirmed. It is worth noting that higher IL-6 levels and its receptor have been found in psoriatic plaques. It seems that obesity has a negative effect on the course of psoriasis, due to the enhanced production of leptin, IL-6, TNF-alpha and decreased synthesis of adiponectin by fat tissue. A negative association between plasma IL-6 and adiponectin levels in obese cases has been shown. Kaur et al. showed a statistically significant increase of IL-6 level in obese cases with psoriasis compared to a control subjects. In cases with psoriasis and with normal body weight, the plasma level of IL-6 was increased in comparison with the control group, although it was not significant. Johnston et al. found similar results. They showed increased levels of IL-6 in obese cases with psoriasis. Another

investigation revealed a positive association between plasma levels of IL-6 and the oxidized LDL-b2-glycoprotein complexes (oxLDL-b2-GPI) in cases with psoriasis. Moreover, a concentration of oxidized LDL and oxidized LDL- b2- glycol- protein complexes proposed a positive association with BMI. According to the investigators, the association between oxLDL-b2-GPI, IL-6 and BMI may propose a link between LDL oxidation and infla

mmation, including the inflammatory mechanisms detected in obesity.

### **Interleukin-8 (IL-8)**

IL-8 is a chemokine which has role in many pathological mechanisms. The main role of IL-8 is a chemotaxis of neutrophils to a place affected by inflammation. It also stimulates antibacterial characteristics of neutrophils. Apart from that, IL-8 stimulates angiogenesis and affects other cells, which take part in inflammatory mechanism including: T lymphocytes, natural killer cells (NK) and basophils, and it is also chemotactic towards keratinocytes. Investigators have drawn attention to the fact that IL-8 has a role in the pathogenesis of type 2 diabetes and of atherosclerosis. Considerably higher circulating levels of IL-8 have been shown in cases with heart failure and metabolic syndrome compared to cases with heart failure without metabolic syndrome. It has been shown that proinflammatory mediators such as TNF-alpha,

IL-1 and CRP stimulate production of IL-8 in human adipocytes. Kobashi et al. showed that IL-8 decreases phosphorylation of AKT by insulin in human adipocytes, which can stimulate resistance to insulin. A significantly increased level of IL-8 has been shown in cases with acute psoriasis. Another investigation revealed a higher IL-8 plasma level in cases with psoriasis, which reduced with the improvement of clinical condition. In an investigation carried out on a Japanese population, a significant increase of serum IL-8 levels in cases with psoriasis was shown, although an association with PASI was not found. The investigators reported increased local production of mRNA for IL-8 or its receptors in psoriatic lesions. To date, there have been no investigations assessing the association between IL-8 levels and the development of metabolic syndrome in cases with psoriasis. However, when the role of this mediator in the pathogenesis of insulin resistance, type 2 diabetes or atherosclerosis is taken under consideration, it cannot be excluded that increased circulating levels of IL-8 in cases with psoriasis may have role in the maintenance of the inflammation, which has a critical role in the pathogenesis of metabolic syndrome.

### **Tumor Necrosis Factor- alpha (TNF-alpha)**

TNF-alpha is a proinflammatory mediator

synthesized by different cells such as: lymphocytes, monocytes/macrophages mast cells and NK cells. TNF-alpha affects the proliferation, activation or differentiation of many cells. TNF-alpha also enhances the production of some proinflammatory cytokines, growth factors and adhesive molecules. Several investigations have reported that TNF-alpha may affect insulin signaling in many cells such as liver, adipose tissue or skeletal muscles. This cytokine may also result in insulin resistance through the inhibition of phosphorylation of tyrosine receptor and insulin receptor substrate 1 (IRS-1). It has been shown that obese mice did not develop insulin resistance if TNF-alpha function had been blocked. Increased concentrations of TNF-alpha have been shown in obese cases. A positive association between TNF-alpha and BMI has also been shown. Recently, it was shown that increased plasma levels of TNF-alpha are correlated with left ventricular diastolic dysfunction, which is one of the earliest presentations of left ventricular dysfunction due to diabetes mellitus, while administration of TNF-alpha inhibitors results in an increase of HDL levels. It has been demonstrated that TNF-alpha is involved in the pathogenesis of metabolic syndrome. TNF-alpha is one of the major mediators in the pathogenesis of psoriasis. Increased levels of this cytokine have been shown in many investigations in cases with active psoriasis. A positive association between serum levels of TNF-alpha and PASI has been reported. It has

been revealed that TNF-alpha levels negatively associated with plasma levels of adiponectin, while this cytokine did not reveal any association with leptin levels in cases with psoriasis. In contrast to other adipokines, TNF-alpha is produced not only within adipose tissue. Therefore, increased levels of TNF-alpha, which are found in the serum of cases with psoriasis, may also caused by the severe inflammatory mechanism in psoriasis as well as from the contribution of other cells in the production of this cytokine. It is interesting that several research investigations have revealed elevated body weight in cases with psoriasis after treatment with anti-TNF-alpha agents.

## 7- PATHOPHYSIOLOGY OF RHEUMATOID ARTHRITIS

### *Genetic Risk Factors*

Rheumatoid arthritis affects about 0.5 to 1% of the population. Ample investigation has been conducted on risk factors for this disease, since it is hoped that this may provide more insight into the involved inflammatory mechanisms and possible opportunities for prevention or management of RA. Several environmental and genetic risk factors increasing disease susceptibility have been found. Twin investigations have revealed that genetic variation accounts for 50 to 60% of the risk on RA development. It has been shown that the HLA-DRB1\*01, \*04, and \*10 alleles are the most important genetic risk factor for RA development, in particular for ACPA-positive RA. Most HLA-DRB1 alleles correlated with RA share an identical amino acid sequence in the peptide-binding groove, which has been known as the shared epitope (SE). The similarity in sequence has resulted in the hypothesis that all predisposing HLA molecules containing the SE sequence may present specific “arthritogenic” peptides, which could result in a joint-specific autoimmune reaction. Given the strong correlation with ACPA-positive RA, it has been proposed that peptides presented by SE-

containing alleles may be citrullinated. It was indeed revealed that conversion of an arginine into a citrulline at the peptide-SE interaction site significantly enhanced the affinity of the peptide for the MHC molecule. Moreover, an investigation focusing on the crystal structure of the HLA-DRB1 antigen complex showed that SE alleles preferentially bound citrullinated peptides, whereas other alleles bound both citrulline and arginine. The high affinity of SE for citrullinated peptides could enhance the amount of HLA peptide complexes on the surface of antigen-presenting cells (APCs), therefore resulting in a (possible joint-specific) T cell response. However, it has proven difficult to find the exact peptide-binding motifs for these SE molecules. Therefore, the exact peptides bound by HLA-SE molecules in vivo remain subject to more evaluation.

Other theories on the role of the SE in RA development have also been proposed, since SE alleles also have another role as a ligand for cell surface calreticulin (CRT), an innate immune receptor present on most human cells and specifically on dendritic cells. The SE-CRT interaction, which is more potent when CRT is citrullinated, is able to stimulate a signal transduction cascade changing the phenotype of the dendritic cell and thereby resulting in skewing of T cell responses to the T helper 17 (Th17) subset and decreased regulatory T cell formation. However, the

exact role of SE-CRT interaction to RA pathogenesis is required to be more evaluated. The different hypotheses on the role of SE in RA are not mutually exclusive, and their relative importance remains unknown.

Besides the HLA region, multiple single nucleotide polymorphisms (SNP) are correlated with rheumatoid arthritis. Among these loci is the PTPN22 gene, the second most important genetic risk factor for RA development. PTPN22 produces a protein tyrosine phosphatase (PTPs), which has role in T cell and B cell antigen receptor (TCR) signaling. Therefore, it may not be surprising that this gene is correlated with multiple autoimmune disorders. Recently, the PTPN22 risk allele was also associated with hypercitrullination of peripheral blood mononuclear cells (PBMCs), a process conducted by PAD enzymes. The association between PTPN22 and both hypercitrullination and T and B cell receptor signaling offers new investigation opportunities to gain more insight in the complex events taking place during the preclinical stage of RA.

### **Autoantibodies in RA**

It is estimated that 50–80% of RA cases harbor autoantibodies. As stated above, the presence of autoantibodies has allowed the detection of subgroups of RA cases that are not only more homogenous with regard to risk factors but also with respect to the clinical disease course. RF,

an autoantibody directed against the Fc part of human IgG, was the first autoantibody system to be reported in RA. The presence of RF was noted so characteristic for RA that it was included in the 1987 ACR classification criteria for RA, despite its suboptimal specificity. Several years later the more RA-specific ACPA were reported. In the ACR-EULAR 2010 classification criteria for RA, both RF and ACPA have been considered. More recently, antibodies against other posttranslationally modified proteins, i.e., carbamylated and acetylated proteins were detected. Seropositive RA is correlated with increased radiographic progression and joint injury, while seronegative RA cases have higher inflammation parameters at presentation. Moreover, not only positivity for a single autoantibody but rather the conjoined presence of multiple autoantibodies may be relevant for characterizing distinct phenotypes of RA cases. Autoantibodies not merely provide effective data on disease outcome but also offer insights into the development of RA. Investigations on the different autoantibodies and their features has resulted in better understanding of the underlying pathophysiological mechanisms in rheumatoid arthritis.

### **Complement Activation**

Another main effector role of antibodies is complement activation. The complement system

can be activated via three mechanisms: the classical pathway (initiated by C1q), the alternative pathway (initiated by C3), and the lectin pathway (initiated by mannose-binding lectin (MBL)), which all result in opsonisation, formation of the membrane attack complex and chemotaxis. In synovial fluid of RA cases, complement levels are decreased, while complement cleavage products are elevated, suggesting increased complement activation. It has been reported that ACPA have the ability to recruit complement via both the classical and alternative mechanisms, but not via the lectin process. Consequently, the evidence proposes that ACPA have the potential to augment the immune response in RA by both Fcγ receptor binding and complement stimulation.



## **8- ROLE OF BIOMARKERS IN RISK ASSESSMENT, DIAGNOSIS, RESPONSE TO TREATMENT, AND PROGNOSIS OF RHEUMATOID ARTHRITIS**

### ***Rheumatoid Factor***

Although RF is basically of the IgM class, it can be of other isotypes, directed against the Fc portion of another Ig, generally IgG. Classically correlated with RA, RF is detected in the serum of approximately 70% of RA cases, and is statistically associated with a worse prognosis, since increased levels of RF are correlated with the presence of aggressive disease, rheumatoid nodules, and extra-articular presentations. However, the diagnostic importance of RF determination alone is limited. Up to 30% of RA cases are seronegative for RF, and this rate can exceed 50% in the early phase of the disease. The RF test shows low sensitivity and specificity. Subjects who do not suffer from arthritis can also present RF seropositivity; among such subjects, the prevalence of RF seropositivity ranges from 5% to 40%, increasing with age. RF may also be present in different other disorders, rheumatologic or not. Therefore, a negative test for RF does not exclude an RA diagnosis, and a positive test should be carefully

interpreted according to the clinical evaluations. In RA cases, changes in the titers of different RF isotypes have been shown over the course of treatment, particularly in investigations evaluating the effect of specific treatments on RF titers. Swedler et al. showed continuous reductions in the titers of three RF serotypes in cases being managed with gold salts. Previously, Lemm et al. showed that IgG RF titers are thought to be a good parameter for evaluating RA activity in cases managed with gold salts. Bobbio-Pallavicini et al. assessed RA cases being managed with infliximab and stated that, although the rate of RF-positive cases remained stable over time, the median RF titer reduced progressively. The same investigators subsequently showed that cases managed with a combination of infliximab and disease-modifying antirheumatic drugs (DMARDs) presented early and significant reductions in IgA RF and IgM RF titers, although not presenting a reduction in IgG RF titers. Moreover, the reduction in IgM RF titers was sustained. Bos et al. investigated a cohort of 188 consecutive cases diagnosed with RA and managed with adalimumab. The investigators associated relative alterations in the levels of IgM RF with the response criteria established by the European League Against Rheumatism (EULAR), and with changes in the acute-phase markers (ESR and CRP). The investigators also showed that the reduction in IgM RF levels was accompanied by a reduction in ESR and CRP values, proposing that IgM RF can act as a

marker of inflammatory function. Different findings were achieved by Ates et al., who investigated 62 cases with RA, assessing RF isotypes (IgA, IgM, and IgG) in terms of their association with RA activity and severity. The investigators reported that, although cases with active RA presented significantly higher levels of IgA RF and IgM RF than did those with inactive RA, the multivariate analysis showed that neither IgA RF nor IgM RF was independently correlated with disease severity. They found that the efficacy of the RF isotypes in evaluation of RA severity was limited. Albeit controversial, the evidence to date proposes that the changes in RF titers is not effective as a marker of disease severity. Therefore, multiple determinations of RF titers are unnecessary during the evaluation of RA cases.

### ***Anti-citrullinated Protein/ Peptide Antibodies***

Recently, different ACPAs have emerged as important tools for the diagnosis of RA. In comparison with RF, ACPAs show similar sensitivity and superior specificity. Moreover, it is possible that ACPAs contribute to the pathophysiology of the disease. The role of ACPAs as markers of RA severity is controversial.

### ***Anti-cyclic Citrullinated Peptide Antibodies***

Among the known antibodies to antigens of the filaggrin–citrulline system, anti-cyclic citrullinated peptide (anti-CCP) antibodies had the greatest clinical efficacy. Anti-CPP determination is a test with high sensitivity (70–75%) and specificity (up to 99%—ELISA II) for RA. It is particularly applicable in RF-negative arthritis cases in the early stage of the disease. Anti-CCP determination can assist in the evaluation of undifferentiated inflammatory arthritis and can enhance the prognostic importance of RF. Since concentrations of anti-CCP antibodies are detectable quite early in (and increase throughout) the course of RA, they can be applied as biomarkers of the severity and prognosis of the disease. Different questions remain regarding anti-CCP antibodies and their role as possible markers of RA severity. In a subanalysis of the Swedish TIRA project, Svärd et al. showed that RA was significantly more active in cases who were positive for IgA anti-CCP than did those who were negative. Regarding the concentrations of IgG anti-CCP, RA activity also tended to be higher in cases who were positive for IgG anti-CCP, although no statistically significant difference was found. Bos et al., evaluating a cohort of 188 consecutive RA cases managed with adalimumab, evaluated IgM RF and the relative changes in anti-CCP levels in comparison with the EULAR response criteria, as well as with changes in acute-phase markers (ESR and CRP). Unlike what had been shown for IgM RF, the investigators showed that the reduction



in inflammatory marker levels was not associated with anti-CCP positivity or with changes in anti-CCP levels, which led them to propose that anti-CCP antibodies may be qualitatively stable RA markers, unrelated to the severity of the disease. Serdaroglu et al., evaluating 40 RA cases and 38 fibromyalgia cases, showed that anti-CCP positivity did not associate significantly with ESR, CRP, analog visual scale scores, DAS28 score, or radiographic results. In short, there thought to be as yet no evidence of an association between anti-CCP levels and RA severity.

### ***Other Antibodies Targeting the Filaggrin–citrulline System***

Other antibodies targeting the filaggrin–citrulline system have also been investigated, such as anti-keratin antibodies and anti-perinuclear factor, anti-filaggrin antibodies, and antibodies to citrullinated fibrinogen. Generally, these antibodies have good specificity for the diagnosis of RA, although their sensitivity is inferior to that of anti-CCP antibodies. There is no finding that fluctuations in the concentrations of these markers associate with RA severity.

### ***Matrix Metalloproteinases***

Matrix metalloproteinases (MMPs), especially MMP-3, are considerably expressed in the rheumatoid synovium of cases with active RA. It has been proposed that serum levels of MMP-3

may constitute a useful marker for the diagnosis and prognostic assessment of early RA. Jensen et al. reported that, in cases with early RA and undifferentiated polyarthritis, serum levels of MMP-3 and pyridinoline varied according to disease severity, proposing that these markers can complement conventional markers in the evaluation of disease severity in RA cases. Posthumus et al., in a prospective investigation of 33 cases with early RA, also concluded that serum levels of MMP-3 were associated with other markers of disease severity such as swollen joint count, ESR, CRP level, and DAS28 score. The same investigators subsequently approved these findings and proposed that MMP-3 and CRP also serve as markers of radiographic progression. Green et al. reported that baseline serum levels of MMP-1 and MMP-3 associated with disease severity. The investigators revealed that such levels were predictive of the functional and radiographic prognosis in untreated early arthritis.

### ***Hyaluronic Acid***

In 82 cases with early RA, Majeed et al. assessed the levels of hyaluronic acid (HA), which have been revealed to be high in RA cases. The investigators assessed how well serum HA levels associate with clinical and laboratory markers of disease severity, i.e., tender joint count, swollen joint count, the Health Assessment Questionnaire (HAQ) score, ESR,

and CRP level. The investigators reported that HA serum levels associated with clinical and laboratory markers of disease severity in early RA and added that, despite the association reported, it was unlikely that HA evaluation would be used in routine clinical practice.

### **Beta C-telopeptide**

Beta-CTX, a marker of bone resorption, has been revealed to present a strong association with the presence of erosions, at baseline and after 2 years, in RA cases. However, this association is thought to be comparable to that found between the presence of erosions and other existing markers. Moreover, beta-CTX does not seem to associate with disease severity.

### **Antibodies to Glycosaminoglycans**

In a group of RA cases, György et al. investigated the natural autoantibodies to carbohydrates, specifically glycosaminoglycans, carbohydrate components of proteoglycans that are secreted in large amounts from degraded cartilage. The investigators reported that the levels of anti-chondroitin sulfate IgM antibodies were inversely associated with both DAS28 score and CRP level in RA cases. The investigators reported that the natural autoantibodies to glycosaminoglycans, antibodies that are extremely abundant and prone to cross-

reaction, can behave as markers of disease severity in RA.

### **Human Cartilage Glycoprotein 39**

Human cartilage glycoprotein 39, also known as YKL-40, is produced by chondrocytes, synovial cells, macrophages, and neutrophils. Some investigations have shown that YKL-40 is an autoantigen in RA. Morgante et al. proposed that YKL-40 is associated with disease severity in RA. Johansen et al. showed that YKL-40 may be a useful tool for evaluating disease severity and improving understanding of the pathophysiology of RA. Matsumoto and Tsurumoto reported that serum levels of YKL-40 only partially reflected the degree of inflammation, and in fact associated with the degree of joint destruction in cases with RA.

### **Mannose-binding Lectin**

It has been shown that low concentrations of mannose-binding lectin (MBL) can be predictive of poor prognosis in cases with early RA. Graudal et al., evaluating findings from 685 Caucasian RA cases in Denmark, showed that low serum levels of MBL, also considered as mannan-binding protein (the complement-activating serum lectin), are correlated with higher ESR as well as with swollen joint count, impaired joint mobility, and annual increase in radiographic destruction score, proposing that MBL

DR. ATOUSA MOGHADAM FARD

levels are markers of RA severity and prognosis.

ROLE OF BIOMARKERS IN RISK, DIAGNOSIS, RESPONSE TO T...

## 9- PATHOPHYSIOLOGY OF CROHN'S DISEASE

### *Pathogenesis and Inflammation*

Crohn's disease pathogenesis is based on tissue inflammation, resulted by an unrestrainable immune response against luminal bacterial antigens. Immune cells like CD4 T-Cells, CD8 T-Cells, B-Cells, CD14 monocytes and natural killers, have role in this mechanism as they infiltrate the gut of CD cases. Part of the immune-mediated susceptibility to CD resides in some innate process of defense form infectious disorders and the intestinal mucus secretion is part of those. It has been revealed that variants of the Muc2 gene decreasing mucus synthesis are correlated to CD in a mouse model. Furthermore, molecules that are mediating bacterial adhesion have been associated to the disease. This is the case of FUT2, which encodes for the fucosyltransferase enzyme, responsible for the production of soluble forms of the ABO antigens. Cases harboring a FUT2 variants reducing the production of the antigens, have a changed interaction with bacteria and are more prone to developing CD. The pathogenesis is also sustained by the interaction of these cells with integrins, adhesion molecules and multiple chemokines, responsible for the synthesis of increased levels of

inflammatory mediators, representing the target of immune and non-immune cells and the promotion of mucosal inflammation. As such, among many adhesion molecules, some results on the involvement of the leucocyte MAdCAM-1, receptor for the  $\alpha 4\beta 4$  integrin, is thought to have a pivotal role. Together with leucocyte adhesion, the role of the extracellular matrix on their activation has been investigated. Proteins like CD44 and CD26 where suggested to have a role as well as metalloproteins (MMP), being MMP1 and MMP3 abundant in the granular tissue close to CD sites of inflammation, therefore responsible for leucocytes stimulation. In the mucosa of CD cases, a dysregulation of different components of the immune system is invariably identified. The most important change is the hyperactivity of T cells with excessive synthesis of mediators, between which IL-12 and IFN- $\gamma$ , promoting a  $T_H1$  lymphocytic phenotype, opposed to the  $T_H2$  one, associating to ulcerative colitis. Furthermore, TNF- $\alpha$  synthesis has also been shown to increase the number of CD4+ FoxP3+  $T_{REG}$  cells, especially in the mucosa of children affected by CD. The inhibition of the effector mediators, like TNF- $\alpha$ , attenuates the detrimental effects in subsets of CD cases. Moreover, the expression of the interleukins, a subgroup of cytokines implicated in the increased or inhibition of other mediators in many different regulatory mechanisms such as maturation, growth and responsiveness of immune cells population, is to be noted anomalous in CD

cases. More analysis of T cell subsets has shown the presence of  $T_H1$  and  $T_H17$  cells in CD, whereas the mediators noted more involved are TNF, IL12 and IL23. Apart from the cited mediators, IL-34 has also been correlated with IBD and CD in particular. IL34 expression is more important in the areas of active inflammation, especially in CD, and is thought to trigger TNF- $\alpha$  and IL6 expression through a ERK-mediated mechanism. Furthermore, IL-34 has been reported as a stimulator of CCL20 through the interaction with its receptor the M-CSFR1, considerably expressed in the inflamed colonic epithelium but not in the healthy subjects. On the contrary, IL-25 inversely associates to the inflammatory state of the cases with IBD, being decreased in CD cases as opposing to healthy controls, and being decreased in the affected areas of the colon if compared to the surrounding adjacent normal tissue from the same cases. Among all the possible interleukins correlated to CD etiology, IL-12 and IL-23 represent the target of still inappropriate treatments because of potential side effects, such as enhanced risk for infection, and the blockade of specific immunological targets, capable of induction of alternative signaling or homing mechanisms. The latter process may also partially clarify the frequent lack of response to treatment with biologics such as Infliximab (Remicade®), a chimeric monoclonal antibody prescribed to manage autoimmune disorders, that works by binding to TNF- $\alpha$  making a decrease in IL-34 expression, implicated in

monocyte and macrophage differentiation, survival and activity.

Although T-Cells are the main effector lymphocytes in intestinal tissue inflammation, also humoral immune system has a pivotal role. Plasma cells differentiation indeed is triggered by CD4 T-Cells, through a process that is firmly dependent on IL-2, overproduced in CD patient's gut. IL-21 converts naive B-Cells into B-Cells expressing granzyme-B: it has a cytotoxic function in the intestinal mucosa and perpetuates the epithelial injury. These proofs suggest that a changed balance between effector and counter regulatory factors probably has role in the sustainment of the tissue-damaging immune response in CD.

Gut microbiota also has a crucial role in designing the inflammatory response in IBD and especially in CD. There is growing findings that some microbial gene products can affect gene expressions in the host. The complex network arising from this assumption is referred to as the microbial-correlated molecular pattern (MAMP) which is sensed by toll-like receptors on immune cells, contributing to their stimulation in the context of the chronic inflammation. Microbiome moreover represent a source of potential pathogenic inputs that can be approached through the techniques applied in the omics era, such as metagenomics investigations, also affecting on our knowledge on geographical variations on the clinical

presentation of the disease. The inflammation is generally transmural and, on pathology evaluation, granulomas may be found on biopsies, with a discontinuous distribution along the longitudinal axis. This inflammatory mechanism often results in irreversible tissue injury in the form of intestinal stenosis or fistulas, inflammatory masses or intra-abdominal abscesses. Cases can develop one or more of these disease behavior and they often tend to evolve from inflammatory to penetrating or stricturing disorder.

## **10- ROLE OF BIOMARKERS IN RISK ASSESSMENT, DIAGNOSIS, RESPONSE TO TREATMENT, AND PROGNOSIS OF CROHN'S DISEASE**

### ***Calprotectin***

Calprotectin is a calcium-binding complex protein that have two subunits. It is considerably expressed in neutrophil and inflammatory monocytes/macrophages, although, under specific situations, its expression has been found in several cell types, including epithelial cells, endothelial cells, fibroblasts, keratinocytes, and osteoclasts. Calprotectin is found in a large variety of fluids, such as human plasma, urine, and cerebrospinal fluid, as well as in saliva. It acts numerous biological activities, including immunoregulation, oncogenesis, and inflammation; proposing the proinflammatory functions mainly triggered by activated granulocytes; and is involved in leukocyte recruitment and cytokine production in inflammatory districts. Calprotectin has also been proposed to have role in protection against pathogen infections, as revealed by experiments indicating that epithelial cells expressing calprotectin are more resistant to bacterial



infections than epithelial cells that do not express calprotectin. The evaluation of fecal calprotectin protein is frequent in clinical practice, and this measure is regarded as a surrogate marker for endoscopic disease severity and gives clinicians some idea of patients' intestinal inflammatory condition without conducting an endoscopy. In acute-phase inflammatory mechanisms, calprotectin is detectable in increased amounts and is associated with increased concentration of CRP.

A recent investigation has shown saliva calprotectin as a potential index of active IBD. Calprotectin levels in stimulated whole saliva are up to three times higher in cases with IBD compared to healthy subjects, and the saliva calprotectin level is higher in stimulated cases with IBD compared to the healthy subjects. However, while it is elevated in both unstimulated and stimulated cases with CD, in cases with UC, it only enhanced in stimulated samples. A possible clarification for these differences between CD and UC may be due to the tissue changes present in the oral mucosa of cases with CD compared to UC. Calprotectin, also considered as the migration inhibitory factor related proteins 8 and 14, is an acute-phase protein with a role in the modulation of neutrophil migration, and its level associates with neutrophil migration and reflects the inflammation level in IBD. Taken together, calprotectin in saliva could be applied as a prognostic marker, as well as an index correlated with the effectiveness

of treatment. However, clinicians must take into account that calprotectin production is also affected by oral inflammation, obesity, oral candidiasis, and periodontal disorders.

### Cytokines

The progress of investigations in recent years has underlined the role of inflammatory cytokines, as it reveals their pivotal role in IBD pathogenesis in terms of modulating the initiation, augmentation, and perpetuation of IBD. Cytokines are also directly play a role in mucosal injury in both CD and UC. The association between levels of salivary cytokines and their amount in plasma has been assessed by some researchers. Twenty-seven cytokine biomarkers have been found in the saliva of healthy subjects, although only IL-6, IFN- $\gamma$ , and MIP-1 $\beta$  revealed an important association with the plasma concentrations, suggesting that salivary cytokine concentrations are affected by the oral environment and the effect of local immunity but, also, by diurnal patterns, although a strong association between saliva inflammatory mediators and their levels in the serum is largely present in healthy, as well as obese and diabetic, subjects and inflammatory disorders in children and adults. Despite the action mechanisms of cytokines not being entirely known, several investigations have shown their involvement in the pathogenesis of IBD. The role of cytokines in UC pathogenesis is well-



known. An atypical T-helper (Th)2 immune response is present in UC, with high concentrations of the proinflammatory cytokines in addition to IL-6, IL-10, and IL-13, whereas CD4<sup>+</sup> lymphocytes with the Th1 phenotype predominate in cases with CD, known by the synthesis of IFN- $\gamma$  and IL-2. The use of salivary cytokines as biomarkers of the inflammatory condition in active and nonactive IBD is known as one of the new frontiers in the follow-up of these pathologies. The investigations performed on salivary cytokines to date agree that IL-6 may be a marker for IBD. In a recent investigation on the rate of caries in cases with CD, IL-6 and tumor necrosis factor (TNF)- $\alpha$  have been reported as increased in the saliva of patients compared to controls. In the latter investigation, saliva was collected, and the salivary flow rate, pH, and IL-6, and TNF- $\alpha$  concentrations were evaluated. The salivary IL-6 and TNF- $\alpha$  levels in cases with CD were higher than in the controls and high in the subgroup of cases with a higher decay-missing-filled index (DMF-T). They also showed higher DMF-T and salivary flow rates in cases with CD compared to the controls, whereas the pH was lower in cases with CD than in the controls. The statistical analysis revealed a positive association of CD duration, CRP, and IL-6 and TNF- $\alpha$  levels with the DMF-T. The investigators therefore assumed that the enhancement in both IL-6 and TNF- $\alpha$  levels may be due to different factors, including their local production by the

inflammatory cells of the oral mucosa and increased synthesis by the inflammatory cells present in infected dental pulp during the caries mechanism. The increase in inflammatory mediators in the saliva in cases with CD is line with a previous prospective investigation by Szczeklik and colleagues. In this investigation, cases with active and nonactive disorders were enrolled and compared to healthy subjects in order to assess whether salivary levels of IL-1 $\beta$ , IL-6, and TNF- $\alpha$  are correlated with the function and oral symptoms of CD, such as oral plaques. The findings revealed that oral plaques characteristic of CD were present in cases with the active disease, whereas nonspecific oral plaques were detected in the groups of both active and nonactive CD. The cytokine analysis in saliva showed a significant enhancement in the levels of IL-1 $\beta$ , IL-6, and TNF- $\alpha$  in active CD compared with inactive CD and the controls, which was associated with the presence of characteristic oral plaques. Higher levels of these proinflammatory mediators were also associated with clinical and biochemical markers of the disease severity. The findings for increasing the IL-6 levels in saliva approved the previous findings by Nielsen and colleagues, who showed that IL-6 was higher in the saliva of both cases with UC and CD than in the controls. They also revealed that the plasma IL-6 of cases with CD associated significantly with CRP and albumin, as well as in UC, where salivary IL-6 was shown to be associated with the IL-6 serum levels.

They reported that the levels of IL-6 is directly involved with the gastrointestinal tract and the mouth cavity, and the IL-6 salivary levels may therefore be known as an additional method for investigating and assessing the disease's severity.

## 11- ROLE OF BIOMARKERS IN RISK ASSESSMENT, DIAGNOSIS, RESPONSE TO TREATMENT, AND PROGNOSIS OF OTHER AUTOIMMUNE DISEASES

### *Systemic Lupus Erythematosus (SLE)*

#### *Erythrocyte Sedimentation Rate (ESR) and SLE*

The ESR has long been considered to be regularly increased in active SLE. It is thus logical that an increased ESR is included in three out of five validated SLE activity scores. In principle, increases in ESR can either be due to alterations in serum proteins or to changes in erythrocytes. The former commonly include hypergammaglobulinemia, monoclonal gammopathy and enhanced fibrinogen levels. The latter mostly reflect decreased erythrocyte number and erythrocyte size. Some of these ESR-accelerating mediators are inflammatory, others are not. To understand the inflammatory component in the enhanced ESR, it is therefore essential to look at all of these components.

### *C-reactive Protein and SLE*

CRP is the standard biomarker of inflammation, but in SLE cases, CRP is more of a biomarker for severe infections. It is therefore of interest to investigate the role of CRP in SLE in some detail. CRP is directly driven by IL-6, and IL-6 levels are elevated in active SLE. Actually, CRP is often not entirely normal in active SLE. Higher CRP levels are identified in cases with active serositis, arthritis, or myositis. In most other conditions, however, CRP concentrations will remain below 60 mg/L or 6 mg/dL in active SLE. Levels higher than these are much more probable in severe infections. CRP concentrations of 150 mg/L or 15 mg/dL make infections very likely, while 20 mg/L (2 mg/dL) CRP or less make infection unlikely. This is of clinical significance given that severe infections are a major cause of death in SLE cases. Of interest, while CRP concentrations, without infection, are higher in active SLE than in inactive SLE, the opposite is true in infections in SLE cases. Accordingly, cases with severe infections have a trend towards lower concentrations of CRP if they have active SLE than if their SLE is inactive. One could argue that this was a outcome of immunosuppression. This, however, is unlikely given that this process is limited to SLE and may other connective tissue disorders, but not identified in ANCA related vasculitis or giant cell arteritis. Therefore, it is much more possible that SLE function affects on the synthesis of CRP. One

hypothesis in this regard is an effect of type I interferons, which seems to decrease IL-6 signaling, similar to the fact that a majority of cases with active SLE show an interferon signature.

### ***Cytokines and Chemokines and SLE***

In addition to the above mentioned routine mediators, a wide variety of cytokines are clearly correlated with SLE severity. Among those are the type I interferons, and IFN $\alpha$  in particular, IL-6, IL-10, IL-15, IL-18, BAFF/BLyS and TNF. While IFN $\alpha$  is mainly produced by plasmacytoid dendritic cells (pDC), the others of these mediators are mostly derived from monocytes and macrophages. In contrast to these, T cell cytokines that act more locally, such as IFN $\gamma$ , can commonly not be assessed in sufficient quantities. Among the cytokines enhanced in SLE, IL-10, IL-15 and BAFF/BLyS predominantly have an immunoregulatory rather than an inflammatory effect.

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