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Bahroz Abbas Mahmood
Department of Microbiology, College of Veterinary Medicine, University of Sulaimani, Sulaimanyah, Kurdistan Region, Northern Iraq.

Correspondence
Bahroz Abbas Mahmood
Department of Microbiology, College of Veterinary Medicine, University of Sulaimani, Sulaimanyah, Kurdistan Region, Northern Iraq.

Evaluate the use of biomarkers of infectious diseases

Bahroz Abbas Mahmood

Abstract
Biomarkers are biological constituents that can helps in detecting of pathological conditions and normal physiological process in the body in approximately precise manner in preclinical studies such as cellular or animal modules particular biomarkers can be monitored. Biomarkers can be used in drug development through replacing endpoints to substitute for clinical points that permits for early evaluation of the drug advantage/effectiveness This work includes hypothesis and interpretation of using biomarkers in detection pathological conditions in three main biological abnormalities, first; Biomarkers used for detection of abnormalities result from autoimmune diseases second; Biomarkers used to detect abnormalities in blood caused by infectious agents third; Biomarkers used to detect abnormalities in CNS caused by infectious agents.

Keywords: Biomarkers, interleukin 2, anti ccp antibodies, HBsAg, TNF alpha.

Introduction
Biomarkers are substances that can help in indicating pathological conditions and normal physiological process in the body (Biomarker definition group, 2001) [6]. Biomarkers can describe many common analyses, measurement of physiological situations. In drug discovery, biomarkers can be used for a variety of applications from target corroboration through developing drug of choice of pathological conditions and clinical trial assessment, regarding to success in gene expression and protein analyte. Protein analyte targets can be available easily in several biological fluids such as serum, saliva, urine or cerebrospinal fluid (Eck, 2010) [17]. In order to useful application, although there are experimental confirmation of the accuracy, strength and limitations of biomarkers it is critical that biomarker candidates be thoroughly capable through a defined discovery and validation process that includes experimental confirmation of the precision, robustness and limitations with deep understanding of the physiological relevance of the marker (Wagner, *et al*, 2010) [45]. Moreover classical biomarkers are able to measure changes in blood pressure, lactate level of blood after exercise and blood glucose level in diabetic patient. While, specific molecular alterations of a cell on RNA, DNA, metabolite and protein level can be mentioned as molecular biomarkers and can be divided into 3 comprehensive groups 1. Those that track disease development over time and associated with known clinical measures 2. Those that determine drug efficacy 3. Those that act as replacement end points in clinical traces. While studies on all of these categories have been done by researchers, biomarkers are used by biotechnology and pharmaceutical companies as drug detection tools to assist in finding of new target in therapeutic involvement and to detect biological response to experimental drugs (Jain, 2010) [26]. In spite of the hope for biomarkers and the development that made, much challenge deceits ahead. Statistically, total number of biomarkers of interest might be expected to be ~1133000, of which approximately accounts 25000 to 30000 are genome, transcriptome 100 000, proteome 1000 000, and metabonome about 2500 to 3000 (Harrigan, 2006, Dettmer, 2004) [23, 15].

2. LPS, interleukin-2 and anti CCP antibody biomarkers in autoimmune diseases
2.1 LPS and interlukin-2 biomarkers in Epstiein-barr Virus
In a research work by Petrara *et al*. in 2012 on Epstein - Barr virus load and immune activation in Human Immunodeficiency Virus type 1-infected patients discussed that, a hallmark of HIV-1 pathogenesis, may play a critical role in the genesis of B-cell lymphomas

besides immunodepression, chronic immune activation (Douek, 2009) [16]. They reported that activation of cells determined by HIV-1 antigen and impaired immunosurveillance against EBV might leads to chronic B-cell stimulation, expansion of EBV-infected B-cells and increase the risk opportunity of progressing EBV-related malignancies. However the factor that may participate in activation of HIV-1-enhanced B-cells is still not fully understood, they predict interleukin (IL)-2 receptor expression is largely stimulated in B lymphocytes of HIV-1-infected subjects, and IL-2 may directly stimulate in vitro propagation of B-cells (David, 1998) [14]. In addition to several IL-2-induced cytokines, such as tumour necrosis factor (TNF) α and IL-6, that may stimulate proliferation and activation of B-cells (Macchia, 1993 and Mauray *et al*, 2000) [31]. Following damage of intestinal mucosa LPS, which is a microbial product are released into circulation and may result in B-cells activation because of great HIV-1-induced reduction in gut (Brenchly, 2006) [9]. The relationship between biomarkers of chronic immune activation and EBV load HIV-1 infected subject were performed in research study by Petrara *et al* in 2012 has observed that the level of LPS and inflammatory cytokines are lower in patients with distorted plasma viremia than in HIV type 1 patients, including those with low levels of plasma viremia under highly active antiretroviral therapy (HAART) and those with CD4-guided therapy interruption. The vital association between activated B-cells and EBV-DNA load detected in a subgroup of patients supports the idea that growth of EBV-infected B-cells arranged by immune activation, particularly, immune activation may help B cell expansion, regardless to EBV infection (Grulich, 2002) [21]. It is expected that expansion of EBV-infected B-cells can be encouraged by immune activation and B-cells binding to HIV-1 either directly or indirectly to HIV-1 can be used as biomarker in patient with HIV-1. Although most of the mechanisms by which HIV-1 activates B-cells probably include proinflammatory cytokines and microbial products produced after cellular/tissue damage, binding of HIV-1 envelope protein to B-cell membranes may activate a polyclonal immunoglobulin class switch recombination (He, 2006) [24].

2.2 Anti CCP antibodies biomarkers used for detect of Rheumatoid arthritis

A study by Allart in 2006 has demonstrated that biomarkers are integral to disease stratification and hence targeted therapy. They have great role in potential changing the management of RA by performing several helpful points such as (i) early diagnosis, (ii) measurement the severity of the diseases, (iii) treatment strategy and intensive care of response to therapy in a considerable proportion of RA patients (Allaart, 2006) [4]. Practically, although delay in diagnosis, and hence therapeutic intervention, is frequently resulting in increased tissue damage, even with early diagnosis pathological change in cartilage and bone has already begun. In patient with undifferentiated RA, Biomarkers are necessary to allow earlier diagnosis. The detection of autoantibodies against cyclic citrullinated peptides (anti-CCP antibodies) in last 10 years ago regarded the most significant progress in the diagnosis of RA (Van, 2009) [41]. Anti-CCP antibodies could be involved in the pathogenesis of RA (Khun, 2006) [29]. Although the diagnostic sensitivity of anti-CCP antibody positivity in cohorts of early synovitis has been described to range between 40% - 71% (Goldbach, 2000 and Visser, 2002) [19], which may be due to lack of anti-CCP development in approximately 30% of RA patients, the specificity of anti-CCP in diagnosis of RA is higher than specificity of traditional RA biomarkers (Lee, 2003). During asymptomatic phase of RA, biomarkers actively required to predict the onset of the disorders. Recent study has proved that the presence of anti-CCP antibodies in patients with RA could assist in predicting the onset of RA. A study by Nielen *et al* in (2005), blood sample were collected from individuals with early stage of RA in retrospective analyses, after examination they detect that anti-CCP might be found in those individuals 9 years before the appearance of clinical symptoms (Nielen, 2004 and Rantapaa, 2003) [34, 36]. However it is estimated that in high epidemiological countries in 5-16% of general population the positive predictive value is much higher than countries with low risk outbreak of RA. A recent study has reported that RA will develop in 69.4% within 5 years in individuals with ≤ 2 first-degree relatives to individuals with RA in presence of anti-CCP antibodies. The recent founding of prospective cohort studies, such as SERA (Studies of the Etiology of Rheumatoid Arthritis) (Kolfenbach, 2009) [28], registering first-degree relatives of probands with RA is expected to facilitate both; calculation of the predictive value of anti-CCP antibodies and other biomarkers and investigation into the natural history of RA and. That development with improvement in cost and toxicity of anti-CCP antibodies can precede the onset of clinical RA by several years highlights the requirement for precise biomarkers that deliver temporal information to detect the actual time of developing of asymptomatic individuals to RA. However, relation between anti-CCP and pathophysiology of RA has not confirmed by evidences, anti-CCP has several advantages as biomarker by; 1.direct correlation and high specificity of anti-CCP in progressed and severe stage of RA, 2. The majority of IgG1 anti-CCP directly associated to effector mechanisms involving complement activation and/or the engagement of Fc gamma receptors (Goronzy, 2003) [27].

3. HBsAg, TNF alpha and IL-8 biomarkers in diagnosis of blood transfusion disorders.

HBsAg biomarkers in hepatitis B virus

Hepatitis B virus (HBV) is regarded as a significant cause of transfusion transmitted infections among blood borne infectious diseases. Blood transfusion problem still appear to be uncontrollable infectious disease particularly in developing countries. The technique of screening accepted by blood bank and its prevalence in community is play a key role in Transfusion related HBV safety and the more sensitive the screening technique accepted the less the chance of transmitting HBV infection through the transfusion (Apetrei, 1996) [1]. All activities to lowering the risk of transfusion transmitted HBV and/or HCV infection play an important role in prevention of this condition. HBV can be detected by biomarker hepatitis B surface antigen (HBsAg), which is regarded as a powerful biomarker for HBV diagnosis (Weber, 2005). HBsAg, which is an enveloped protein of hepatitis B virus (HBV), is a glycosylated lipoprotein can be seen in large amounts in the serum of infected individuals, it has a spherical shape with diameters of 22 nm or filaments of similar diameter (Vyas, 1978) [44]. HBsAg is one of the first serum biomarkers to observe throughout the course of HBV infection and can be detected

14-56 days prior to biochemical indication of liver dysfunction and the beginning of jaundice and it is cleared within a few months in self-limiting illness. While persistence of HBsAg for more than 6 months, spontaneous clearance is very unlikely and individual who have this condition is considered as chronic HBV carrier in screening of blood donors, HBsAg detection is routinely used and An HBsAg negative donation is generally considered safe. Because of antigen-antibody interaction dependant of HBsAg diagnostic assay, they are susceptible to mutations and HBV may be transmitted from HBsAg negative donors. (Carman, 1997; Weber, 2005). A study by Waters in 1992 has demonstrated that HBsAg in blood donors can be detected by several assays such as ELISA and Elecs HBsAg is highly sensitive and specific and represents a major improvement over the alternative assays for the detection of HBsAg in blood donors and in routine laboratory diagnostics. Future developments of serological assays should include monoclonal antibodies that identify epidemiologically related surface antigen mutants and further optimization of sensitivity. Although, technology for nucleic acid testing in blood screening laboratories has not yet been developed, alternative would be blood donors testing with amplification of nucleic acid. The disadvantages of these procedures are mainly linked to the complications associated to the sample automation processing and to the potentials of cross-contamination of samples because the sensitivity of amplification is very high (Waters, 1992) [46].

3.1 TNF alpha and IL-8 biomarkers in Sickle cell anaemia

Several factors such as the environment, origin, social, economic and genetic factors identified by clinical heterogeneity is regarded as predisposed factors associate with congenital recessive autosomal blood disorder in sickle cell anaemia (SCA). The associations between SCA and BetaS-globin haplotypes such as presence of. 37-kb deletion in alpha2- thalassimia (a2-thal3.7 kb) together with concentration of fetal haemoglobin (prognostic biomarker) regarded as a genetic factor in individuals with SCA (Buchanan, 2004) [10]. The betaS-globin gene haplotypes can be seen in patients with SCA and it t correlates with the clinical features. In general, two types of haplotypes are associated occurrence of symptoms which in presence of CAR haplotype patients presented with more severe symptoms, while in the presence of SEN haplotype the symptoms is mild and the prognosis is better (Nagel, 2001) [32]. In spite of a common genetic background, there is a variation in phenotypic appearance in individuals with SCA, that ranged from minor complication, with likely prolonged survival rate (60–70 years of age) to very severe clinical symptoms with multi-organ dysfunction and early mortality (Adams, *et al*, 2003) [2]. Tumour necrosis factor alpha (TNF-alpha) and interleukin-8 (IL-8) are regarded other biomarkers, which they can be used as diagnostic tools in patients with SCA and they have roles in stimulating of leukocyte, endothelial cell and stimulation of macrophages (Asiss, 2005 and Abbas, 2007) [5, 3]. In patients with SCA, increased level of IL-8 indicate poor prognosis based on the relation of IL-8 to an elevation of S haemoglobin and decrease in F haemoglobin in red blood cells because of an increase in intravascular haemolysis and increases in oxidative damage, cellular activation, vascular occlusion and consequently inflammation (Figueiredo *et al*., 1996) [18]. In patients with SCA, during steady state and crises events the level of TNF-alpha and IL-8 in serum is increased (Lanaro, 2009) [30] and they are likely involved in vascular obstruction events. Cytokines imbalance in patients with SCA are important in presenting clinical sings (Pathare, 2003) [35]. Moreover, inter-patient variations of the level of cytokine may be recognized to gene polymorphisms, particularly in the A alleles of -308 G>A and _251 A>T, which are positioned in the promoter regions of the TNF and IL-8 genes, respectively, and have been associated with higher TNF-alpha and IL-8 transcript levels (Hacking *et al*, 2004) [22]. Based on these observations, recent study has investigated that TNF- alpha and IL-8 genes and their contribution with levels of cytokines and medical history are regarded as a classical biomarkers in patients with SCA.

4. Biomarkers in central nervous system diseases

4.1 Microvesicles biomarker for diagnosis of CNS disease

Most types of cells release micorviscles (MVs), which derived from endothelial cells or platelet during physiological conditions, in addition to pathological condition like; cellular activation or neoplastic transformation (Mallat *et al*., 2000; Jung *et al*., 2012). This feature makes MVs have a role in detection of pathological conditions by providing information about types of activated cells and on the nature of the activation. Moreover detection of MVs from neural cell origin may be very helpful in patients with neuroinfection, especially in those cases were the pathogen may be indefinable, since it is known that MVs are carriers for infectious agents, and isolation of MVs from the CSF may help to increase significantly the sensitivity of available tests. In tissues that cannot access easily for direct examinations such as CNS, MVs are very useful in detecting abnormalities and they can be used as both strong biomarker or as an implement to examine the biology of these tissues. It has been observed that there is relation between increased release of MVs and acute or active phase of neurological disorders and it can be used as a very useful marker to support suitable treatment in neurological pathologies with a vascular or ischemic pathogenic component. A study by Ahn and co-workers in 1990 has observed that MVs that derived from platelet stained for CD42 and identified by FACS are increased in plasma of individuals with ischemic stroke, particularly in individuals with temporary ischemic attacks, in comparison to those with large vessels thrombosis. (Lee et al., 1993). However, In this study no correlations had been drawn with the extent of the ischemic area, or the severity of the outcome but after 10 years the results have been confirmed by using CD61 and CD62P instead of CD42 to identify MVs of platelets origin, in whole blood of patients with ischemic stroke (Cherian *et al*., 2003; Pawelczyk *et al*., 2009). They therefore start to use the rate of MVs derived from platelet as a biomarker to be used in a population at risk to have a stroke, to detect individuals with higher chance, or basically close to develop the event. Due to the limitation in measurement of platelet derived MVs, studies in Japan by (Shirafuji *et al*., 2008; Kuriyama *et al*., 2010), have used ELISA as alternative for quantification of platelet marker CD42 or CD42a on ultracentrifugated plasma MVs. They have found direct relation between ischemic stroke and occurrence of these markers. In addition to platelet, MVs can be derived from endothelium and it is more promising in identification of CNS diseases such as in cerebral ischemia. Endothelium-derived MVs recently identified and linked to cerebral ischemia, may be more

promising. A study by Simak in 2006 has reported the number of endothelial MVs in plasma which is stained with FACS for CD105, CD144, phosphadityl serine (PS), and CD54, with number of parameters such as stroke size, severity and prognosis of the patients. It has been observed, the amount of lesion directly associated with the number of $CD144^+$ MVs which they expect transforming of haemorrhage of the ischemic lesion and indirectly associated with number of $CD105^+$, $CD54^+$, PS^+. (Simak *et al*., 2006). One year later a study by Williams in 2007 has observed conflicting results , by detecting similar $CD31^+$ or $CD62E^+$endothelial MVs levels acute ischemic stroke patients and in stroke mimics (Williams *et al*., 2007), this contrasting result thought to be due to several technical limitations, including the use of archival samples stored frozen for over 1 year. The original finding have been confirmed by Jung and co-workers in 2009, they have detected significant association between elevated of MVs derived from endothelium to stenosis of both intra and extra portions of cerebral arteries. Moreover, they specified their observation by associating separate MVs markers for extra-cranial ($CD62E^+$) and intra-cranial ($CD31^+CD42b^-PS^+$) localization of the stenosis and the positive correlation between number of plasma endothelial MVs and size of infarct and degree of severity. According to analysis of expected parameters, in patients with high risk factors, levels of endothelial MVs inversely associated with the duration of ischemic stroke occurrence (Jung *et al*., 2009). In patients with inflammation of neuron , the role for MVs may be more difficult to define, since solid biomarkers, such as MRI, are already available, and microglial MVs in the CSF, despite holding promise, may remain a non-specific parameter, helpful but not critical to make diagnosis and difficult to use for monitoring. The predictive value of endothelial MVs known as ($CD105^+PS^+$, $CD62E^+$, or $CD106^+$), has been established in a different clinical setting, namely the risk to develop cerebral vasospasm in patients with spontaneous subarachnoid haemorrhage (Lackner *et al*., 2010), in which, MVs derived from platelet may play a role. Recently, in treatment of patient with ischemic stroke during acute phase, biomarkers have an important role in treatment strategy and reduce therapeutics side effects. Interestingly, neural MVs molecules vary form a particular disease to other and may be carry different molecules in each phase of particular disease (Bianco *et al*., 2009).

4.2 Biological biomarkers for diagnosis and monitoring response of neoplastic meningitis

In detecting neoplastic meningitis (NM), several biomarkers in cerebro spinal fluid (CSF) have been increased such as β_2-microglobulin, lactic dehydrogenase, αfetoprotein, and human chorionic gonadotropin (Boehem *et al*,. 2007 and Yoshida, 2005) [7, 47], but due to low sensitivity of them, their use has been limited (Chamberlain, 2006) [13]. Additionally, without enough knowledge about biology of metastatic disease in general and brain metastasis in particular, the clinical importance of these biomarkers is limited (Groves, 2003) [20]. Having enough information about the biology of disease development and the molecular processes of tumor metastasis can be helpful in detect new biomarkers with high sensitivity and specificity for NM that also could be used as replacements of CSF tumor burden and response to therapy (Brandsma *et al*. 2006 and Roy *et al*,. 2008) [8, 37]. Preferably, early diagnosis and knowledge about prognostic value to select therapy can be monitored by detecting these biomarkers. At time of metastasis, there is an increasing in motility and invading ability of cells of primary tumour, which allowing them to penetrate capillaries, venules and lymphatic channels such as extravasation of tumor cells of CNS by migration through endothelial and basement membrane to reach the CSF and meninges. In patients with NM some molecules which involved in this process such as matrix metalloproteinases (MMP) and cathepsins, have been raised. In CSF of patients with metastatic brain tumor and positive cystic cytology, MMP-2 and MMP9 which they have ability to digest portions of the extracellular matrix have been detected (Stockhammer, 2000) [40]. In addition to those biomarkers, in patients with NM Other proteases (cathepsin B and H) and protease inhibitors (cystatin C) can be used as a useful indicators. In comparison to patients with cancer without CNS diseases, it has been detected that the level of cathepsins B and H have been elevated and the level of cystatin C concentration has been reduced in the CSF of patients with NM (Nagai, 2003) [33]. Studies have observed that, molecules linked to tumor cell tropism for specific organs such as chemokine biomarker (CXCL-8, CXCL10 and CXCL18), also may be useful for diagnosing and monitoring NM. Research study by Brandsma and co-worker in 2006 have found that in presence of CNS disease, levels of CXCL8, CXCL10 and CXCL18 could be precisely detected when assayed together with protein and glucose levels. In metastatic process of neoplastic tissues, molecules which involved in angiogenesis are also important. Even though angiogenesis has not been recognized as significant for development of NM, several molecules involved in angiogenesis like in vascular endothelial growth factor (VEGF), urokinase-type plasminogen activator, tissue-type plasminogen activator (tPA), and antithrombin III) seems to have indicative importance. In patients with NM, levels of VEGF have been demonstrated in their CSF, in comparison to cancer patients without NM (Van *et al*, 2006 and Reijneveld *et al*., 2005) [43, 38]. On the other hand, the diagnostic specificity and sensitivity and the threshold VEGF concentrations used vary from study to study, for example, Herrlinger and co-worker have detected a relatively high 98.3% of diagnostic specificity but a relatively low sensitivity of 51.4% for NM using threshold VEGF levels of 250 pg/mL (73 % sensitivity for a threshold of 100 pg/mL) (herrlinger, 2004) [25]. Correspondingly, in three other recent studies, low sensitivity (50%–69%) but high specificity (100%) using a threshold VEGF level of 20pg/mL was observed. Another study recognizing reductions in tPA in the CSF of patients with NM, which used a combination index of the log of VEGF and tPA, achieved a sensitivity of 100% but a substantially lower specificity (73%) than detected in previous VEGF studies. Although the diagnostic utility of VEGF remains to be established, some studies have suggested that it has predictive value. Herrlinger *et al* in 2004 has detected that VEGF levels reflect the clinical course in some patients, appearing markedly decreased following therapy and increased upon relapse. Similarly, Stockhammer and co-workers re reported a reduction in CSF VGEF levels in 80% of patients with different types of primary cancer in response to treatment. However, additional broad study is necessary, by the time tracking of VEGF levels, may demonstrate a useful tool for monitoring the response of NM to therapy. Careful consideration must be done during developing new diagnostic tests, because, use of

CSF biomarkers in this process is subjected to several inherent problems. Additionally, in patients with NM CSF flow abnormalities are common, and uneven CSF flow can change the concentration of biomarkers in low flow areas, resulting in highly variable measurements at different sites of sampling (Chamberlain, 1991) [11]. Moreover changes in the level of biomarkers can be seen during sampling of CSF from a lumbar versus a ventricular source depending on the location of metastases along the neuraxis (Chamberlain, 2001) [12]. Although the more generic markers of malignancy (eg, VEGF) may tolerate for more consistency, different tumor histologies are likely to be associated with different levels of certain biomarkers and the ideal CSF sampling time is also necessary, because molecular expression may change with disease development.

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