Neutrophil to lymphocyte ratio and immature granulocyte: assessing for promising parameters to monitor tuberculosis-diabetes mellitus patients

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ABSTRACT

Tuberculosis remains as a major global public health threat and infected more than >10 million cases worldwide. Nowadays, public have witnessed epidemiological shift between chronic and infectious disease globally. Diabetes mellitus as a non-communicable disease and on the other side, Tuberculosis as an infectious disease coexist in the same individual may became health challenge in the near future. DM’s impact on clinical presentation and treatment outcome of TB remains poorly. Detecting and managing TB patients with DM comorbidity by routine laboratory screening provides an opportunity for monitoring patients’ prognosis and decreasing disease severity to better outcomes. But in facts, not all laboratory services can provide complex yet expensive assays. Studies has shown Neutrophil to Lymphocyte Ratio (NLR) and Immature Granulocyte Percent (IG%) may be an option as an easy, quick, simple, low-cost, repeatable and reliable assays to monitor TB-DM patient’s prognosis.

Keywords: tuberculosis, diabetes mellitus, neutrophil to lymphocyte ratio, immature granulocytes, laboratory

INTRODUCTION

Tuberculosis (TB) persists as a major global public health threat and the leading causes of death worldwide. Based on projections, approximately TB has infected about a quarter of global population. More than >10 million cases in 2022 and >1.6 million death were reported globally. 30 High TB Burden Countries contributes in 80 % TB cases and it remains challenge for health authorities to achieve “End TB” strategies in 2030 [1].

Recently, public have witnessed development and progression of new public health burden includes two different diseases: Tuberculosis (TB) as an infectious disease and on the other hand Diabetes mellitus (DM), as a non-communicable disease. This new syndemic progression is sky rocketing and represent a health challenge in the near future [2]. The epidemiological shift occurring when both chronic and infectious illnesses coexist not only in the same population but also in the same individual is exemplified by comorbidity of TB and diabetes [3].

International Diabetes Federation (IDF) reported >500 million people are currently living with diabetics and it is estimated this number will increase gradually to 783 million diabetics globally in 2045 [4] due to lack of physical activity, not abundance fibre and poor diets. Although diabetes mellitus (DM) is primarily urban, but it is also becoming more prevalent in semi-urban and rural areas, often undiagnosed and complicated by another complication [5]. DM develop alterations in the immune system, increasing susceptibility risk to Mycobacteri-
um tuberculosis infection, as well as drug resistance risk, treatment failure, morbidity and recurrent disease [6,7]. Prospective cohort study reported 18% TB patients had diabetes and these individuals had worse outcomes than non-diabetic pulmonary TB patients [8].

World Health Organization (WHO) jointly with International Union against Tuberculosis and Lung Disease (IUALTD) have recommended the “bi-directional” screening and integrated management for TB-DM disease in high burden countries. But in low and middle-income countries, health system and management frequently undependable and not adequate to respond those dual burden diseases [7]. The incidence and worsening prognosis of TB-DM patients may become higher year by year if controlling and monitoring efforts are not implemented. Detecting and managing TB patients with DM comorbidity by routine laboratory screening provides an opportunity for monitoring patients prognosis, decreasing disease severity to better outcomes [5]. Maintaining optimal disease outcomes and minimizing toxicity, drug interactions, and other issues when managing TB-DM optimally is crucial yet challenging.

Mostly scientific literature focuses on the TB-DM prevalence, with barely any kind of evidence to help clinicians manage TB-DM patients clinically. Management and strategies to prevent diabetic patients infected with TB and vice versa is important, but we cannot neglect following-up patients that already diagnosed by TB-DM. Routine laboratory assays can be the choice for monitoring TB-DM patient’s prognosis and infection level yet providing data for clinicians to obtain the best treatment. But it remains challenge choosing the most quickly, low-costly, and easily obtained result. White blood cell count (WBC) is a well-known assay to evaluate inflammation. Inflammatory response is valuable to the TB pathophysiology [9].

Immature Granulocytes percent (IG%) recently being studied and able to be a marker for sepsis diagnosis and more indicative than other clinical parameters such as C-reactive protein, and IL-6 [10]. TB patients have lower lymphocyte counts, higher neutrophil and higher monocyte counts also stimulatingly increasing neutrophil immature number as define by “left shift” or increasing neutrophil immature divided by total granulocyte [11,12]. Neutrophil to Lymphocyte Ratio (NLR) is emerging as a new marker of inflammation in many diseases including lung diseases [12,13]. Both NLR and IG% are automatically provided by Hematology analyzer through routine blood count. It does not need an additional workload and cost yet easily repeatable [12]. This article discusses about NLR and IG% as an easy, low cost and promising routine laboratory as say to follow up TB-DM patient’s inflammation prognosis.

**TB-DM PATHOPHYSIOLOGY AND OUTCOME**

Diabetes Mellitus is a serious, chronic disorder with major adverse effects on people’s lives, families, and societies and remains top 10 causes of death in adults [4]. DM characterized as hyperglycaemia caused by non-sufficient insulin production, insulin resistance, or both [7]. T2DM (Type 2 Diabetes Mellitus) contribute at least 90% whole DM cases worldwide. All DM types are progressive diseases that lead into many complication aftereffects.

DM patients show compromised and alteration of innate immunity leading dysfunction of neutrophils, macrophages, Natural Killer (NK) and any other cell components [14]. Immunity disorder can be affected by high-level glucose level leading into Advanced Glycation End Product (AGE) products. Inflammation is vital for host response in order to fight against pathogens that infected the body. As a response for *M. tuberculosis* infection, cytokine secreted by innate immune cell and adaptive immune cell collaborate to eliminate microorganisms [15]. Diabetic macrophage has increased CCR2 expression and reduced CD14 receptor expression, which contributed to restrain monocytes migration to the lung and also reduced MTB phagocytosis. Cytokines such as IL-1β, IL-6 and TNF-α production are lower in diabetes patients compared to healthy subjects. It also reported an association between glycaemic control and cytokine production [16].

TB-DM patient are more infectious when diagnosed, as they appeared to have a higher bacterial load also more likely to have pulmonary cavities and haemoptysis [14]. Uncontrolled glycemic level increasing infection risk including *M. tuberculosis* infection, worsening prognosis also increasing risk of multi-drug resistance TB. DM increased the risk of death in individuals with active tuberculosis and relapse risk after treatment completion. Another study stated that patient with TB-DM were reported to have other comorbidities. A person with DM is 1.8 to 9.5 times apparent to be infected with TB compared to non DM individual [14].

**TB-DM PATIENTS MONITORING MANAGEMENT AND CHALLENGE**

World Health Organization suggests performing any collaborative care to TB-DM patients with collaborative health services focused in to three aspects: (1) Establish a strong collaborative services mechanism (2) detecting and monitoring TB in DM patients (3) detecting and monitoring DM in TB patients (bi-directional care). Routine screening labo-
ratory assay for DM in TB patient and vice versa, routine screening TB for DM patient (bi-directional screening) are likely to give another chance for early diagnosis, better prognosis follow-up and treatment management plan in the future.

Glycemic control in DM patient is to maintain glycated haemoglobin (HbA1c) <7% throughout the treatment. But in facts, this target seems hard to obtain because in early phase of TB infection, active TB will initiate hyperglycaemia. On the other hand, Rifampicin also interact with DM drugs leading into decreasing drug performance [6]. Glycemic control and strategies for minimizing the risk of cardiovascular disease are part of diabetes care during antituberculosis treatment which aims to improve TB treatment outcome and reduce DM-related morbidity and mortality [17]. Maintaining Glycemic level control itself cannot represent whole patients’ state of health. It needed another laboratory findings to assess the disease severity. Hematology assay itself is likely the most preferable assay in medical field due to its simple, easy, quick and repeatable. While discussing about infection disease, White blood cells count is dependable to represent the patient state.

NEUTROPHIL TO LYMPHOCYTE RATIO AS A PROGNOSTIC BIOMARKER

Neutrophil is the first cell which response bacterial infection also dominate acute inflammation phase. This cell also has a leading function in innate immunity before adaptive immunity is formed. Neutrophil is well-known broadly as a pivotal cell in the defense against bacterial infection and can eliminate bacteria effectively because of their huge stocks of proteolytic enzymes and quick synthesis of Reactive Oxygen Species (ROS). This cell also releases a web-like structure called neutrophil extracellular traps (NETs) to immobilize and eliminate microorganism like bacteria [18]. Pro-inflammatory cytokines and chemokine modulator immunity are secreted by neutrophil to initiate another cell recruited into the infection site. Activated neutrophil contains many enzymes and antimicrobe molecules leading to kill microbes [19].

Abnormal number of blood neutrophils counted from full blood count is cheap, fast and ubiquitous laboratory finding for inflammation assessment. Neutrophil to Lymphocyte ratio (NLR) has attracted attention as a new inflammatory marker [18]. It is calculated easily form total neutrophil count derived by total lymphocyte count. NLR can represent both innate and adaptive immunity system by Neutrophil and Lymphocyte [19]. High level NLR can indicate in the course of chronic inflammation. Therefore, NLR is more stable and less influenced by pathological and physical factor than another leucocyte assay.

Several studies reported NLR usefulness result on the prognosis markers of several diseases such as lung diseases [20] [21], diabetes and cardiovascular disease [22] [23], cancer [24] [25] and sepsis [26]. NLR in early stage would help identify adverse outcome and observation plan in Community Acquired Pneumonia [13]. High NLR ≥ 5 were associated with pulmonary cavitation yet increased severity of inflammation also increased risk of mortality and exacerbation in Chronic obstructive pulmonary disease (COPD) OR: 2.9 [27]. Another study stated that NLR can support in differentiation of tuberculosis and sarcoidosis [12]. Cut-off value of NLR for one-year mortality in miliary tuberculosis is 5.2 and NLR ≥ 5 remarkable decreased in survival rate [28].

NLR also been used in DM care and management control by many studies. Meta analysis conducted by Adane and colleagues confirms that NLR value was significantly associated with poor glycemic control in T2DM patients (OR=1.50 (95%CI: 1.30-1.93)) [29]. NLR can be used as a predictive prognosis in Diabetic foot ulcer patients to undergo amputation and another implication. Calculating NLR is easy and elevating number of NLR can predict worsening inflammation in diabetic nephropathy patient. Cardiovascular disease (CVD) risk is increased in DM patient and increased NLR can be used to predict CVD in T2DM patients [30].

Both TB and DM condition regularly required monitoring to achieve a better outcome. Offering NLR as a regular assay procedure is very promising. Elevated NLR physiologically shows an inability of immune system to suppress infection [25]. NLR suggest a low-cost, non-invasive, quick and early opportunity to assess TB-DM patient prognosis and status plus allow physicians to make better decision about treatment and therapy.

IMMATURE GRANULOCYTE AS A PROGNOSTIC INFECTION BIOMARKER

During infection, mature neutrophils are proliferating to kill bacteria. Then, immature neutrophils will enter the bloodstream. This “left-shift” infection response is defined as an increased ratio of immature granulocytes to total granulocytes [10]. Immature granulocytes themselves consist of mainly promyelocytes, myelocytes and metamyelocytes but not include band form neutrophils [31]. Immature granulocytes percentage nowadays is automatically assayed and calculated by hematology analyzer. It does not require any other reagents, additional workload, low cost yet repeatable by routine blood count.

Normal IG% in healthy population may be varying (0.0-0.1%). Diabetes patients tend to develop any other disease besides TB, such as cardiovascular
complication, renal disease, etc. [32]. In cardiovascular disease, IG% were assessed as a prognostic value and predict risk of mortality [33]. In lung disease management, IG % can be useful as a predictive marker of COVID-19 [34] and distinguish severe COVID-19 patient [35]. Elevated IG% found to be correlated with elevated C-reactive protein and proc-alcitonin than healthy or control group. It is associated with an acute-phase response in which activated bone marrow to release IG into bloodstream [36]. Several studies conducted with IG% as a marker shows in the table below (Table 1).

Increased IG% in the peripheral blood is directly related to systemic inflammation intenseness. This cell can be founded when bone marrow is stimulated during bacterial infection, trauma and sepsis. Early response of IGs is promising to be an indicator of decreasing immune response and inflammation severity. It may be more accurate than total white blood count [38]. Elevated IG% may be an indicator for tuberculosis severity infection.

### NLR AND IG AS A PROMISING TB-DM PROGNOSIS

The number of interests regarding to biomarkers and biosignatures for TB active detection and monitoring is encouraging. But in facts, only a small proportion of those markers are suitable in many kinds of laboratory and health service providers. TB-DM as a progressive yet infectious disease is required a regular follow up and treatment strategies to reduce worsening prognosis and mortality throughout the treatment. Immunological and biomolecular assays are dependable but requires complex and expensive technology. Biomolecular assays are promising to predict adverse outcomes of pulmonary TB. Thus, in limited health services with limited human resources and limited laboratory settings cannot accommodate these complex assays to be done.

Hematological parameters have a crucial role in treatment strategies and monitoring treatment of TB-DM cases. It can influence patient’s outcome afterwards. Hematological findings (NLR and IG%) are cost-effective yet able to provide clinically useful information to support management care of TB-DM patient through the treatment to improve treatment outcomes, survival rate and quality of life [39]. CRP (C-reactive protein) is a well-known assay and used widely in many Laboratory services. However, it lacks specificity as an inflammatory biomarker [36]. Nowadays, health services seek a promising assay to provide quick and useful information in order to plan the next treatment and management care. NLR and IG% counts are proved by many studies as a promising and reliable parameter to be a prognostic biomarker in many diseases. Therefore, it needs further studies to determine cut-off value including AUC, ROC, specificity and sensitivity in TB-DM cases.

### CONCLUSION

To manage TB-DM patients, health facility used to give more attention and management care in order to decrease severity, mortality risk, and treatment failure. Patients should be monitor regularly to maintain its condition. NLR and IG% can be the promising assay to TB-DM prognosis assay. Both NLR and IG% count provides quick, reliable, low cost and simple result.

**Conflict of interest**

The authors declare no conflict of interest, financial or otherwise.
REFERENCES


