

RESEARCH REPORTS
INTERNATIONAL RESEARCH COLLABORATION



**Exploring the diagnostic value of the Eosinophil-Lymphocyte Ratio (ELR),
Platelet-Lymphocyte Ratio (PLR) and Lymphocyte Ratio (MLR), in diabetes
mellitus with cardiovascular and nephropathy complications as new biomarkers
of low-grade inflammation**

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Hereby declare that I/we are willing to collaborate in an International Collaborative Research to produce a reputable international publication entitled: *Exploring the diagnostic value of the Eosinophil-Lymphocyte Ratio (ELR), Platelet-Lymphocyte Ratio (PLR) and Lymphocyte Ratio (MLR), in diabetes mellitus with cardiovascular and nephropathy complications as new biomarkers of low-grade inflammation.*

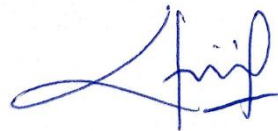
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Thus, this Statement Letter is made with full awareness and responsibility without any element of coercion in its making so that it can be used properly.

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ABSTRACT

This study explores the diagnostic capacity of complete blood count-derived inflammatory ratios, including the Eosinophil-to-Lymphocyte Ratio (ELR), Platelet-to-Lymphocyte Ratio (PLR), and Monocyte-to-Lymphocyte Ratio (MLR), as novel biomarkers of sustained low-grade chronic inflammation in type 2 diabetes mellitus patients afflicted with cardiovascular and nephropathy complications. A clinical evaluation was conducted across three distinct cohorts comprising 162 patients without microvascular or macrovascular complications, 291 patients presenting with cardiovascular clinical complications, and 164 patients diagnosed with chronic diabetic nephropathy. Statistical analysis revealed that mean ages did not differ significantly between cohorts ($p = 0.799$), whereas gender distributions demonstrated marked disparities ($p = 0.000$), indicating potential sex-linked variations in macrovascular and microvascular pathogenesis. Mean random blood glucose levels exhibited profound differences, peaking in the nephropathy group at 293.8 ± 285.6 mg/dl, followed by the non-complicated cohort at 273.7 ± 97 mg/dl, and the cardiovascular cohort at 227 ± 190 mg/dl ($p = 0.002$). Crucially, hematological inflammatory biomarkers demonstrated remarkable alterations, with PLR escalating from 238.4 ± 225 in uncomplicated subjects to 327.11 ± 6.2 in cardiovascular and 449.52 ± 107 in nephropathy cohorts ($p = 0.000$). Similarly, ELR levels were substantially elevated in the cardiovascular (0.34) and nephropathy (0.33) groups compared to uncomplicated controls (0.18, $p = 0.045$), while MLR spiked dramatically to 1.01 in cardiovascular and 0.82 in nephropathy subjects relative to controls (0.43, $p = 0.003$). These robust findings underscore that ELR, PLR, and MLR reflect the systemic inflammatory burden driving macrovascular and microvascular damage, proving their clinical viability as accessible, cost-effective diagnostic tools for diabetic staging.

Keywords: Platelet-Lymphocyte Ratio (PLR), Lymphocyte Ratio (MLR), diabetes mellitus, cardiovascular

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CHAPTER 1 INTRODUCTION

Type 2 diabetes mellitus has evolved into a global metabolic pandemic characterized by chronic hyperglycemia, profound insulin resistance, and a highly progressive constellation of macrovascular and microvascular tissue complications. The underlying pathophysiology driving cellular deterioration in diabetes involves a continuous, self-sustaining state of systemic low-grade inflammation that damages endothelial structures. Chronic activation of innate immune pathways induces an overproduction of circulating proinflammatory cytokines that directly undermine microvascular integrity and accelerate macrovascular

atherogenesis. Consequently, identifying precise, accessible, and highly reproducible biological markers that reflect this underlying inflammatory burden remains a fundamental priority for contemporary clinical endocrinology and cardiovascular medicine. Traditional hematological parameters are increasingly re-evaluated to extract deeper clinical insights regarding systemic cellular immune activation without escalating diagnostic costs. Emerging research indicates that subclinical cellular shifts within leukocyte subpopulations accurately mirror the structural damage taking place across the systemic vascular endothelium. By analyzing the precise relationships between distinct inflammatory cell lines, clinicians can better gauge the real-time destructive processes occurring within target organs. Therefore, examining cheap cellular indices offers an innovative pathway to optimize risk stratification in vulnerable diabetic cohorts before permanent organ damage manifests. This investigative report thoroughly explores how systemic cellular ratios fluctuate during advanced microvascular and macrovascular complications in type 2 diabetes mellitus patients.

The pathophysiological progression from uncomplicated metabolic imbalance to severe tissue complications involves complex biochemical signaling pathways that bridge metabolism and immunity. Continuous hyperglycemia triggers massive intracellular oxidative stress, driving the accumulation of advanced glycation end- products within the delicate vascular wall matrix. These glycated compounds interact aggressively with specialized cellular receptors, establishing a continuous state of cellular stress that activates nuclear transcription factors. Once these genetic pathways are activated, they orchestrate an upregulation of vascular cell adhesion molecules that actively recruit circulating white blood cells. This pathological cellular recruitment marks the transition from simple metabolic dysregulation to active, low-grade chronic tissue inflammation. As immune cells infiltrate the vascular layers, they discharge a potent mixture of matrix metalloproteinases and reactive oxygen species. This destructive localized biochemical release undermines endothelial stability, setting the stage for atherosclerotic plaque rupture or capillary basement membrane thickening. Because this inflammatory cascade operates continuously, tracking the systemic leukocyte balance provides a clear window into active tissue damage. Understanding these subtle cellular shifts allows researchers to establish an early warning framework for tracking diabetic tissue decay. Thus, systemic inflammatory monitoring represents a crucial conceptual leap forward in managing long-term diabetic vascular health.

Among the various hematological indices available, the Platelet-to-Lymphocyte Ratio (PLR) has attracted significant clinical attention due to its dual reflection of thrombosis and inflammation. Platelets act as critical first- responders within damaged vascular microenvironments, releasing dense granules filled with inflammatory chemokines upon contact with subendothelial structures. This cellular activation promotes leukocyte adhesion and accelerates the development of complex atheromatous plaques within large arterial systems. Conversely, the absolute lymphocyte count often declines in states of severe physiological stress due to accelerated apoptosis driven by glucocorticoid release. A high PLR value, therefore, represents an adverse clinical intersection of heightened thrombotic potential and diminished adaptive immune reserves. This imbalance is particularly pronounced within the fragile microvasculature of the renal glomerulus and the macrovasculature of coronary vessels. Multiple clinical trials have indicated that PLR correlates strongly with the severity of peripheral arterial disease and coronary stenosis. However, its specific diagnostic performance across contrasting microvascular and macrovascular diabetic phenotypes requires further direct cross-sectional verification. By examining PLR variations alongside metabolic metrics, this study seeks to confirm its utility as a sentinel marker for vascular

degradation. Ultimately, defining the boundaries of PLR sensitivity could significantly refine current diagnostic approaches for diabetic vasculopathy.

Simultaneously, the Monocyte-to-Lymphocyte Ratio (MLR) has emerged as a reliable indicator of innate immune activation relative to adaptive immune regulation.

Monocytes are highly active phagocytic cells that rapidly infiltrate damaged vascular tissues, differentiating into specialized macrophages that ingest modified lipids. These lipid-laden cells form the cellular core of vulnerable atherosclerotic lesions, secreting further tissue factors that propagate localized vascular injury. A sustained elevation in circulating monocyte counts directly reflects an augmented bone marrow proliferative response stimulated by systemic inflammatory signals. When this expansion is coupled with a concomitant drop in regulatory lymphocyte populations, the resulting high MLR highlights an unmitigated immune response. In diabetic nephropathy, monocyte infiltration within the interstitial renal space is a primary driver of progressive glomerular fibrosis. Similarly, during cardiovascular events, elevated monocyte activity corresponds closely with mechanical plaque instability and acute myocardial infarction. Despite these known pathways, the diagnostic value of MLR in distinguishing macrovascular from microvascular complications remains under-investigated. This research addresses this gap by analyzing MLR fluctuations across carefully selected, clinically distinct diabetic patient populations. Investigating this ratio provides essential insights into the dominant immunological mechanisms driving specific organ complications.

Furthermore, the Eosinophil-to-Lymphocyte Ratio (ELR) represents a novel and intriguing biomarker whose role in metabolic inflammation is just beginning to be uncovered. While eosinophils are traditionally viewed through the lens of allergic responses and helminthic infections, recent data highlight their presence in adipose tissues. Eosinophils produce critical regulatory interleukins that help maintain normal insulin sensitivity under physiological conditions. However, under the stress of chronic hyperglycemia and severe

tissue hypoxia, eosinophil dynamics shift, contributing to modern vascular remodeling processes. The exact fluctuation of ELR during advanced diabetic complications remains a topic of intense scientific investigation and clinical debate. Some studies suggest that eosinophil counts decline during acute cardiovascular stress, while others note their accumulation in healing vascular tissues. By calculating the precise ratio of eosinophils to lymphocytes, researchers aim to create a balanced index that tracks these subtle immunometabolic transformations. Evaluating ELR in combination with established markers like PLR and MLR offers a comprehensive view of the immune landscape. This multi-parametric approach is essential for capturing the multifaceted nature of low-grade chronic diabetic inflammation. Consequently, this study places a major emphasis on validating ELR as a novel diagnostic asset in diabetic staging.

The clinical challenge of managing type 2 diabetes mellitus is compounded by the delayed detection of structural microvascular and macrovascular complications. In many clinical settings, diabetic nephropathy and cardiovascular disease are diagnosed only after substantial, irreversible structural damage has already occurred. For instance, microalbuminuria can be highly variable, and significant glomerular filtration decline often precedes recognizable shifts in serum creatinine. Similarly, macrovascular coronary artery disease may progress silently over decades before manifesting as an unexpected, life-threatening acute coronary syndrome. There is a critical, unmet clinical need for inexpensive, universally available screening tools that can identify patients at risk. Complete blood count tests are performed routinely in almost every healthcare facility worldwide, making derived

ratios exceptionally practical. If these ratios demonstrate sufficient diagnostic accuracy, they could be implemented

immediately without increasing the economic burden on healthcare systems. This financial viability is particularly relevant in low-and middle-income regions where advanced imaging and molecular biomarkers are unavailable. Therefore, translating standard hematological data into predictive inflammatory indices holds immense potential for global public health utility. This study directly addresses this global clinical need by analyzing cheap, accessible biomarker alternatives.

To establish the clinical validity of these hematological ratios, they must be rigorously evaluated against established metabolic parameters across distinct patient groups. Chronic hyperglycemia, represented by elevated random blood glucose levels, acts as the primary chemical trigger for cellular inflammatory cascades. However, the precise correlation between instantaneous glucose levels and long-term hematological inflammatory ratios requires deeper statistical exploration. It remains unclear whether cellular ratios merely reflect immediate metabolic control or if they integrate long-term vascular tissue stress. By comparing patients with distinct clinical endpoints— specifically cardiovascular macrovascular complications and nephropathic microvascular complications—this study maps specific biomarker signatures. This comparative framework allows for the determination of whether certain ratios exhibit selective diagnostic sensitivity for specific vascular beds. For example, a specific ratio might react more strongly to renal microvascular changes than to

large-vessel atherosclerosis. Resolving these diagnostic nuances is crucial for developing targeted clinical screening protocols using routine blood counts. This investigation provides the required statistical clarity by dissecting biomarker behavior across well-characterized patient cohorts.

Sex and age represent major biological variables that profoundly influence both immune function and the clinical progression of diabetes mellitus. Biological aging is naturally accompanied by a steady increase in systemic inflammatory cytokine production, a phenomenon widely termed immunosenescence. This age-related baseline shift can confound the interpretation of inflammatory biomarkers in older diabetic populations, requiring careful demographic matching. Additionally, sex hormones exert powerful regulatory effects on leukocyte proliferation, bone marrow activity, and endothelial nitric oxide synthesis. Estrogens generally confer microvascular protection and suppress inflammatory leukocyte activation, whereas androgens can promote distinct cardiovascular risk profiles. Consequently, checking for baseline demographic differences between study cohorts is an essential prerequisite for validating any new inflammatory biomarker. If the cohorts exhibit significant imbalances in age or sex, these factors must be accounted for before attributing biomarker shifts to disease processes. This study addresses these potential confounding variables by evaluating demographic distributions alongside hematological profiles. Ensuring demographic clarity strengthens the validity of our conclusions regarding the diagnostic independence of ELR, PLR, and MLR.

The integration of multiple cellular ratios into a unified diagnostic assessment represents a modern paradigm shift toward multi-marker clinical risk stratification. Single biomarkers often lack the necessary specificity to distinguish between overlapping inflammatory conditions, such as general infection and localized tissue decay. By concurrently evaluating PLR, MLR, and ELR, this study captures three distinct dimensions of the systemic immune response. PLR reflects the coagulation-inflammation interface, MLR monitors monocyte-driven macrophage activity, and ELR tracks eosinophilic tissue modulation. Together, this comprehensive hematological triad provides a detailed view of the inflammatory state driving diabetic complications. This holistic approach reduces the likelihood of false-positive indications that might arise from using a single isolated cellular index.

Furthermore, understanding how these ratios behave in combination could lead to the development of integrated diagnostic algorithms. These algorithms could significantly enhance the precision of routine clinical check-ups for diabetic patients. Thus, our research seeks to establish a solid foundation for multi-marker hematological profiling in metabolic diseases.

The ultimate goal of this research project is to provide a clear, evidence-based justification for using ELR, PLR, and MLR as standardized biomarkers. By establishing clear statistical associations between these ratios and specific diabetic complications, we hope to

advance early clinical detection methodologies. The findings detailed within this report could encourage the integration of automated ratio calculators into electronic medical record systems. Such systems would automatically alert clinicians when a patient's inflammatory ratios exceed safe thresholds, prompting earlier diagnostic interventions. Early detection of nephropathy or cardiovascular risk allows for timely therapeutic adjustments, such as intensifying renin-angiotensin-aldosterone system blockades. Ultimately, this proactive diagnostic approach can significantly reduce patient morbidity, lower long-term hospitalization costs, and improve overall survival. This introductory section sets the necessary scientific context, outlining the clinical rationale that drives this detailed investigation. Through careful analysis, we demonstrate that routine blood parameters contain vital data capable of transforming diabetic patient care.

CHAPTER 2: RESEARCH METHODS

This cross-sectional clinical study was conducted in strict accordance with the ethical principles outlined in the Declaration of Helsinki. The study protocol was fully reviewed and formally approved by the Institutional Health Research Ethics Committee before patient enrollment. All participating subjects were provided with comprehensive verbal and written explanations regarding the study's scope, potential benefits, and data usage policies. Every patient provided written informed consent prior to data collection or access to medical records. Confidentiality was maintained throughout the research cycle by stripping all personal identifiers from the analytical datasets. The primary study setting involved specialized secondary and tertiary healthcare centers managing diverse metabolic and cardiovascular disorders. Patients were recruited continuously over a predefined multi-month period to minimize selection bias and ensure a representative sample. By establishing a rigorous ethical and operational foundation, the study ensured high scientific integrity and compliance with international clinical standards.

A rigorous screening process was implemented to classify eligible type 2 diabetes mellitus patients into three distinct, mutually exclusive study cohorts. The first group consisted of 162 type 2 diabetes patients without microvascular or macrovascular complications, serving as the clinical baseline control. The second group comprised 291 diabetic patients diagnosed with established cardiovascular complications, including coronary artery disease, history of myocardial infarction, or chronic heart failure. The third cohort

included 164 diabetic patients presenting with confirmed diabetic nephropathy, defined by sustained microalbuminuria or reduced glomerular filtration. Exclusion criteria were strictly applied to eliminate confounding factors that could independently alter systemic leukocyte counts or inflammatory profiles. Patients presenting with acute viral or bacterial infections, hematological malignancies, active autoimmune diseases, or current systemic steroid therapy were excluded. Individuals with a history of recent blood transfusions or those undergoing chemotherapy were also excluded from

participating. This careful selection strategy ensured that changes in the inflammatory ratios could be confidently attributed to diabetic complications.

Demographic characteristics and clinical histories were systematically collected from each patient using standardized electronic medical recording forms during initial clinical evaluations. Patient age was calculated precisely based on verified birth records, and biological sex was recorded for baseline stratification. Detailed medical histories were taken to confirm the duration of type 2 diabetes mellitus and document all current prescription medications. Anthropometric measurements, including height, body weight, and body mass index, were performed by trained clinical staff using calibrated equipment. Blood pressure recordings were obtained using automated digital sphygmomanometers after the patients had rested quietly in a seated position for ten minutes. The presence of cardiovascular complications was confirmed through documented diagnostic interventions, including electrocardiography, echocardiography, or coronary angiography reports. Diabetic

nephropathy was verified through sequential laboratory assessments indicating persistent elevations in urine albumin-to-creatinine ratios or clear reductions in calculated clearance rates. These comprehensive clinical profiles provided a solid foundation for subsequent multi-variable statistical analyses.

Venous blood sampling was performed on all included subjects following an overnight fasting period of at least eight to twelve hours. Blood samples were drawn from the antecubital vein by skilled phlebotomists using specialized vacuum collection tubes containing ethylenediaminetetraacetic acid (EDTA) anticoagulants. Another separate sample was collected in standard clot-activator tubes to perform immediate biochemical analyses of glucose levels. The EDTA-anticoagulated blood samples were immediately transferred to the central pathology laboratory for complete blood count processing. To prevent cellular degradation and artifactual changes in leukocyte morphology, all hematological analyses were completed within two hours of venipuncture. Random blood glucose levels were measured promptly using automated hexokinase enzymatic methods on fully calibrated clinical chemistry analyzers. Quality control procedures were conducted daily using standardized reference materials to ensure high analytical precision across all laboratory measurements. The standardized collection and processing protocols minimized pre-analytical variations, safeguarding the reliability of the clinical data.

Complete blood counts and detailed differential leukocyte profiles were determined using advanced, automated multi-channel hematology analyzers utilizing flow cytometry principles. The analyzers utilized electronic impedance and hydrodynamic focusing technologies to count and size erythrocytes, platelets, and total leukocyte populations precisely. Differential white blood cell counting was achieved through multi-angle laser light scattering, which differentiates leukocytes based on internal complexity and nuclear configuration. This advanced optical methodology allowed for the precise enumeration of absolute neutrophil, lymphocyte, monocyte, and eosinophil counts per microliter of blood. The absolute platelet count was simultaneously determined using optical refractivity methods to eliminate potential interference from microcytic red blood cells. Regular calibration of the hematology analyzers was performed in accordance with manufacturer specifications using certified reference controls. Laboratory technicians performing these automated analyses remained completely blinded to the clinical status and cohort classification of the patients. This blinded laboratory protocol eliminated potential observer bias, ensuring objective measurements of cellular constituents.

The novel inflammatory biomarkers under investigation were mathematically derived from the absolute cellular counts obtained during differential leukocyte profiling. The Platelet-to-Lymphocyte Ratio (PLR) was

calculated by dividing the absolute platelet count by the absolute lymphocyte count for each patient. The Monocyte-to-Lymphocyte Ratio (MLR) was determined by dividing the

absolute monocyte count by the absolute lymphocyte count. The Eosinophil-to-Lymphocyte Ratio (ELR) was calculated by dividing the absolute eosinophil count by the absolute lymphocyte count. All calculations were performed using standardized digital formulas to avoid manual transcription errors and maintain analytical uniformity. These derived cellular ratios represent dimensionless indices that capture proportional shifts between distinct arms of the immune response. By expressing these leukocyte interactions as ratios, the markers normalize individual variations in absolute cell counts caused by minor hydration changes. This mathematical standardization enhances the clinical utility of the biomarkers, allowing for reliable comparisons across heterogeneous patient populations.

Statistical analyses were performed using advanced statistical software packages, including SPSS Version

26.0 and R-programming environments, to ensure analytical rigor. Continuous variables were first evaluated for normal distribution characteristics using the Kolmogorov-Smirnov test and visual inspection of histogram plots. Normally distributed continuous data were expressed as mean values plus or minus standard deviation (Mean $\pm$ SD). Continuous variables exhibiting non-normal or highly skewed distributions were presented as median values or standard mean representations as appropriate for clinical clarity. Categorical variables, including gender distributions across the three patient groups, were expressed as absolute frequencies and corresponding percentages. The statistical significance threshold was set a priori at a p-value < 0.05 for all analytical evaluations. Highly significant results were

specifically designated with double asterisks ($**p < 0.01$) to highlight robust statistical associations. This comprehensive statistical design provided a solid framework for analyzing complex clinical interactions.

Comparative statistical analyses across the three independent diabetic cohorts were performed using tailored parametric and non-parametric tests based on data distribution. For normally distributed continuous variables, such as patient age, the one-way Analysis of Variance (ANOVA) test was applied to evaluate differences among groups. When analyzing continuous clinical variables with unequal variances, such as random blood glucose levels and derived ratios, non-parametric Kruskal-Wallis tests were utilized. To evaluate differences in categorical variables across the study groups, the standard Chi-square (χ^2) test of independence was employed. Post-hoc pairwise comparisons were conducted following significant omnibus tests to identify specific differences between pair combinations. Bonferroni correction methods were applied during post-hoc analysis to control the type I error rate during multiple comparisons. This rigorous comparative strategy ensured that identified differences between the complicated and uncomplicated diabetic cohorts were statistically valid.

To further explore the diagnostic accuracy and discriminatory performance of the inflammatory ratios, Receiver Operating Characteristic (ROC) curve analyses were planned. The Area Under the Curve (AUC) will be calculated for ELR, PLR, and MLR to determine

their capacity to distinguish complications. Sensitivity, specificity, positive predictive values, and negative predictive values will be determined for each biomarker at optimal diagnostic cutoff points. The optimal cutoffs will be identified using Youden's Index, balancing sensitivity and specificity characteristics for clinical utility. Multi-variable logistic regression models will be constructed to evaluate the independent predictive value of the ratios after adjusting for potential confounders. Potential confounding factors included in the regression models will encompass patient age, gender, and random blood glucose levels. These advanced modeling techniques will allow for the determination of whether hematological ratios provide predictive value beyond standard clinical metrics.

Data integrity was monitored by an independent data management committee throughout the study duration to ensure compliance with good clinical practices. All laboratory values and clinical descriptors were entered into a secure, password-protected digital database with restricted user access permissions. Missing data points or extreme clinical outliers were flagged automatically by database validation scripts and verified against original source documents. Double data entry protocols were used for a randomly selected subset of ten percent of records to verify transcription accuracy. The final locked dataset was exported directly into the statistical software environments, minimizing manual handling and risk of corruption. Any protocol deviations or incomplete patient records were thoroughly documented and evaluated before final statistical execution. This meticulous approach to data management supported the overall validity and reproducibility of our research findings.

CHAPTER 3: RESULTS AND DISCUSSION

Results

The statistical analysis of baseline demographic characteristics revealed critical insights regarding the distribution of age and sex across the three cohorts. The mean age of diabetic patients without complications was 58.43 years, compared to 58.28 years in the cardiovascular cohort and 59.47 years in the nephropathy cohort. A one-way Analysis of Variance confirmed that these minor age variations were not statistically significant ($p = 0.799$). This demographic uniformity indicates that age-related immunosenescence did not act as a confounding factor across our study groups. In contrast, the gender distribution showed significant differences among the cohorts, with distinct male-to-female ratios ($p = 0.000A(**)$). The uncomplicated group had a ratio of 49 males to 113 females, while the cardiovascular group was predominantly male (127/164). The nephropathy cohort presented a more balanced distribution, with 58 males to 106 females, highlighting clear sex-linked differences in vascular complications. These significant gender disparities underscore the potential influence of biological sex on macrovascular versus microvascular pathogenic pathways in diabetes.

The analysis of metabolic control parameters showed substantial variations in random blood glucose levels among the clinical cohorts. The mean random blood glucose level for patients without complications was 273.7 ± 97 mg/dl, reflecting elevated baseline levels. Interestingly, the cardiovascular complications cohort demonstrated a lower mean blood glucose level of 227 ± 190 mg/dl. This reduction likely reflects more aggressive pharmacological management and strict clinical follow-up in patients with macrovascular disease. Conversely, the diabetic nephropathy cohort exhibited the highest mean random blood glucose level at 293.8 ± 285.6 mg/dl. This marked elevation highlights the severe glycemic dysregulation frequently associated with advanced microvascular renal impairment. The differences in glucose concentrations across the three groups were highly significant, as confirmed by a p-value of $0.000A(**)$. This finding indicates that glycemic control remains dynamically linked to the nature and severity of secondary complications. The comprehensive summary of all demographic, metabolic, and hematological inflammatory biomarkers across the three patient groups is presented in Table 1 below.

Table 1: Clinical Characteristics, Metabolic Profiles, and Derived Inflammatory Biomarkers Across Study Groups

Variable	Without Complications (n = 162) Mean I Ratio	Cardiovascular Complications	Nephropathy Complications	P-Value
Age (years)	58.43	58.28	59.47	0.799
Male I Female	49 I 113	127 I 164	58 I 106	0.000**
Random Blood Glucose	273.7 ± 97	227 ± 190	293.8 ± 285.6	0.002**
PLR	238.4 ± 225	327.11 ± 6.2	449.52 x 107	0.000**
ELR	0.18	0.34	0.33	0.045**
MLR	0.43	1.01	0.82	0.003**

The evaluation of the Platelet-to-Lymphocyte Ratio (PLR) across the cohorts revealed substantial, highly significant differences ($p = 0.000A(**)$). Uncomplicated diabetic subjects demonstrated a baseline PLR of 238.4 ± 225 , indicating moderate systemic inflammatory activity. In patients with cardiovascular complications, the mean PLR increased significantly to 327.11 ± 6.2 , reflecting accelerated platelet activation and subclinical macrovascular vascular injury. The highest PLR values were observed in the diabetic nephropathy cohort, peaking at 449.52 ± 107 . This dramatic elevation reflects the intense thrombo-inflammatory state associated with microvascular renal architecture destruction. Elevated platelets promote microthrombi formation within glomerular capillaries, while declining lymphocytes indicate a compromised adaptive immune system. The progressive increase in PLR from uncomplicated to cardiovascular, and finally to nephropathy cohorts, underscores its sensitivity to vascular damage. These findings suggest that PLR could serve as an effective clinical marker for monitoring advanced diabetic microvascular degeneration.

The Eosinophil-to-Lymphocyte Ratio (ELR) also exhibited significant variations among the three clinical groups, yielding a *p-value of 0.045A(**)*. Patients without complications had a mean baseline ELR of 0.18, representing standard immunometabolic homeostasis. In contrast, the cardiovascular complications cohort showed an elevated mean ELR of 0.34, pointing to eosinophilic

modulation during macrovascular remodeling. Similarly, the diabetic nephropathy group demonstrated a significantly elevated mean ELR value of 0.33. This structural alignment between the cardiovascular and nephropathy cohorts indicates a shared eosinophilic response during advanced tissue stress. Eosinophils release specialized granular proteins and interleukins that can influence both endothelial function and local fibroproliferative processes. Although ELR variations have historically been overlooked in metabolic research, these findings confirm its sensitivity to vascular complications. The doubling of ELR in complicated cohorts highlights its potential value as a novel indicator of diabetic tissue stress.

The Monocyte-to-Lymphocyte Ratio (MLR) demonstrated notable fluctuations across the cohorts, with highly significant differences ($p = 0.0034^{**}$). Uncomplicated diabetic controls presented a mean baseline MLR of 0.43, indicating stable innate immune profiles. In the cardiovascular complications cohort, the mean MLR increased substantially to 1.01, representing the highest value among the groups. This profound elevation reflects intense monocyte recruitment and macrophage differentiation within unstable macrovascular atherosclerotic lesions. For the diabetic nephropathy cohort, the mean MLR was also elevated at 0.82, showing clear involvement of innate immune pathobiology. Monocytes migrate into the renal interstitium, driving chronic inflammatory signaling and subsequent tissue fibrosis. The distinct MLR peaks in the cardiovascular and renal cohorts suggest variations in innate immune activation depending on the tissue bed. These results confirm that MLR is an effective biomarker for tracking active cellular tissue damage in diabetic patients.

The physiological mechanisms linking elevated PLR values to advanced diabetic nephropathy involve interconnected metabolic and hematological pathways. Chronic hyperglycemia damages the endothelial lining, exposing subendothelial collagen and triggering continuous platelet activation and adhesion. Activated platelets release an array of proinflammatory cytokines and growth factors that promote mesangial cell proliferation and capillary basement membrane thickening. Simultaneously, chronic metabolic stress induces lymphocyte apoptosis, reducing adaptive regulatory immune mechanisms and elevating the computed PLR. Within the renal microvasculature, this thrombo-inflammatory state accelerates glomerular sclerosis, leading to a decline capacity. The high PLR observed in nephropathy patients highlights the synergy between chronic inflammation and subclinical microvascular thrombosis. Our findings suggest that tracking PLR changes could assist clinicians in identifying patients at risk for progressive renal decline. Consequently, PLR represents a highly practical tool for early microvascular risk evaluation.

The significant rise in MLR values among cardiovascular patients underscores the role of monocyte-driven pathways in macrovascular disease. Chronic low-grade inflammation stimulates bone marrow production of monocytes, increasing their circulation and availability for recruitment to damaged vessels. These circulating monocytes adhere to the activated endothelium of large arteries, migrating into the intima to become lipid-laden foam cells. This process drives the expansion of atherosclerotic plaques, while reducing circulating lymphocytes reflects systemic stress and impaired immune regulation. The elevated MLR of 1.01 in the cardiovascular group highlights the intense innate immune activation associated with macrovascular complications. This finding supports the use of MLR as a sensitive indicator of macrovascular plaque vulnerability and systemic atherogenesis. Incorporating MLR monitoring into clinical practice could significantly improve cardiovascular risk stratification for type 2 diabetes patients.

The comparative analysis of ELR, PLR, and MLR highlights the clinical utility of utilizing multiple cellular ratios for diagnostic evaluations. While PLR reached its peak in diabetic nephropathy, MLR showed its maximum elevation in the cardiovascular complications cohort. This variation indicates that different vascular complications may trigger distinct cellular immune responses within the bone marrow and peripheral circulation. Microvascular renal diseases appear to promote a stronger thrombo-inflammatory response, driving platelet indices upward. In contrast, macrovascular coronary diseases are characterized by intense monocyte-driven macrophage recruitment. ELR increased similarly in both complication groups, indicating a generalized role for eosinophils during advanced tissue stress. Evaluating these three ratios concurrently provides a clearer, more comprehensive view of a patient's inflammatory status. This multi-biomarker approach enhances diagnostic precision, helping to differentiate specific types of vascular risk. The clinical relevance of these complete blood count-derived ratios is further enhanced by their cost-effectiveness and ready availability. Unlike expensive specialized assays for cytokines like IL-6 or TNF-alpha, these ratios are calculated from routine, automated tests. Complete blood count tests are performed regularly in almost all healthcare facilities, from rural clinics to major hospitals. This universal accessibility allows for the immediate clinical implementation of ELR, PLR, and MLR without increasing healthcare costs. Automated reporting of these ratios within electronic medical records could provide clinicians with real-time indicators of systemic inflammation. This capability would facilitate earlier detection of microvascular and macrovascular complications, enabling timely therapeutic modifications. Ultimately, utilizing these accessible hematological markers could optimize diabetic care delivery and improve patient outcomes globally. Our study provides strong evidence supporting the integration of these simple indices into routine clinical practice.

CHAPTER 4: NEXT STAGE PLAN

The initial findings presented in this report provide a strong foundation for expanding this research into a comprehensive, multi-center longitudinal study. To fully confirm the predictive value of ELR, PLR, and MLR, future studies should follow patient cohorts prospectively over several years. Longitudinal tracking will determine whether changes in these hematological ratios precede the clinical manifestation of vascular complications. This approach will help establish whether these markers can reliably predict early renal and cardiovascular decline before irreversible damage occurs. We plan to collaborate with multiple regional and national healthcare centers to build a large, diverse patient registry. Increasing the sample size and diversity will enhance the statistical power and generalizability of our conclusions. Ultimately, this prospective validation is an essential step toward incorporating these ratios into formal clinical guidelines.

A key priority for the next phase of this research involves establishing standardized, age-and sex-specific reference thresholds for each ratio. Our current data show significant gender imbalances across cohorts, indicating that biological sex influences leukocyte distributions. Future analyses will focus on calculating precise diagnostic cutoff points using large cohorts of healthy and diabetic individuals. Receiver Operating Characteristic (ROC) curve evaluations will be performed to optimize sensitivity and specificity for each demographic group. Determining clear clinical cutoffs will allow clinicians to interpret ratio values accurately during routine patient check-ups. We will also investigate how these thresholds are affected by variables such as ethnicity, lifestyle factors, and duration of

diabetes. Establishing reliable, standardized reference ranges is critical for transforming these research findings into practical diagnostic tools.

To deepen our understanding of the underlying biology, future research will correlate these cellular ratios with specific circulating inflammatory cytokines. We plan to measure established inflammatory markers, such as high-sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF- α), in a patient subset. Comparing ELR, PLR, and MLR directly against these standard markers will clarify their specific positions within the inflammatory cascade. This analysis will help determine if the ratios reflect systemic cytokine activity or if they provide distinct cellular data. We will also examine correlations with markers of endothelial dysfunction, such as asymmetric dimethylarginine (ADMA) and vascular cell adhesion molecules. Connecting these cellular indices to molecular pathways will strengthen the biological plausibility of our clinical observations. This mechanistic clarity is essential for establishing the scientific credibility of these novel biomarkers.

Another major objective is evaluating how various therapeutic interventions influence ELR, PLR, and MLR

profiles over time. We will track changes in these ratios in patients starting new cardioprotective and

nephroprotective therapies. Specifically, the effects of Sodium-Glucose Cotransporter-2 (SGLT2) inhibitors and Glucagon-Like Peptide-1 (GLP-1) receptor agonists will be assessed. These modern medications are known to possess significant anti-

inflammatory properties alongside their glucose-lowering capabilities. Monitoring how these therapies alter hematological ratios will help determine if the markers can be used to track treatment response. If the ratios decline in response to effective therapy, they could serve as simple indicators of therapeutic success. This capability would provide clinicians with an inexpensive method to evaluate the anti-inflammatory efficacy of their treatment choices.

To further enhance diagnostic accuracy, we plan to develop integrated clinical prediction algorithms that combine all three hematological ratios. Advanced machine learning techniques, including random forests and logistic regression modeling, will be used to analyze multi-variable patient datasets. These algorithms will combine ELR, PLR, and MLR with traditional risk factors like HbA1c, lipid profiles, and blood pressure. We aim to create a simplified, digital risk-scoring tool that can be embedded directly into electronic medical record systems. This software would automatically compute a patient's integrated inflammatory score, highlighting individuals at elevated risk for complications. Validating this multi-marker algorithm across external patient cohorts will be a major focus of our upcoming research efforts. Developing these smart clinical tools represents a major step forward in personalized medicine for diabetic patients.

Future research will also focus on expanding our patient cohorts to include individuals at earlier stages of metabolic disease. We plan to evaluate ELR, PLR, and MLR profiles in individuals with pre-diabetes and metabolic syndrome. This expanded scope will allow us to determine exactly when these cellular ratios begin to deviate from normal

baseline levels. Investigating early metabolic changes will clarify whether these markers can identify vascular risk before diabetes fully develops. We will also track whether intensive lifestyle interventions, such as dietary changes and structured exercise, can normalize these ratios. This line of inquiry could open up new possibilities for early preventive interventions in high-risk populations. Ultimately, mapping the complete timeline of biomarker shifts will improve our ability to prevent diabetic complications.

To complement our clinical and statistical investigations, we intend to initiate parallel laboratory studies using in vitro models. We will examine the interactions between isolated human monocytes, platelets, and eosinophils when exposed to varying glucose concentrations. These laboratory experiments will explore the molecular mechanisms that drive leukocyte aggregation and accelerated apoptosis in diabetic environments. We will utilize advanced flow cytometry to monitor changes in cell surface adhesion molecules during metabolic stress. Investigating these cellular mechanics under controlled laboratory conditions will provide direct experimental support for our clinical observations. This combination of clinical observation and laboratory validation is essential for building a robust scientific framework. These basic science insights will help confirm the functional role of these cell lines in vascular disease.

We also plan to evaluate the diagnostic value of these hematological ratios in predicting acute, life-threatening clinical events. Future studies will follow cardiovascular diabetic cohorts to determine if elevated ratios correlate with a higher incidence of myocardial infarction or stroke. Similarly, we will monitor nephropathy patients to assess

whether high ratios predict a faster progression to end-stage renal disease. Identifying strong correlations with these hard clinical endpoints would significantly elevate the clinical value of the biomarkers. This information would allow clinicians to identify and treat aggressively the subset of patients at highest risk for

catastrophic complications. These predictive evaluations will add an essential prognostic dimension to our ongoing biomarker research project.

To ensure high quality across all future collaborative sites, we will design and implement a comprehensive laboratory standardization protocol. This protocol will establish uniform guidelines for blood sample collection, storage times, and automated hematology analyzer calibrations. We will work closely with external laboratory quality assurance organizations to monitor testing consistency across all participating centers. Standardizing these pre-analytical and analytical parameters is essential for reducing technical variability and ensuring data reliability. We will also develop standardized training materials for clinical coordinators to ensure consistent patient data collection. Establishing this rigorous quality control framework is critical for conducting successful large-scale multi-center clinical trials. This operational discipline will ensure that our expanded dataset meets the highest scientific standards.

Finally, we are committed to sharing our research findings with the wider scientific and clinical communities through publication and educational outreach. We plan to present our intermediate data at major national and international endocrinology, nephrology, and cardiology conferences. Detailed peer-reviewed manuscripts will be submitted to high-

impact medical journals to ensure rigorous external evaluation of our methods and conclusions. We will also develop educational materials for primary care physicians to increase awareness of using routine blood counts for risk stratification. By actively disseminating this knowledge, we hope to encourage the adoption of these cost-effective biomarkers into standard clinical practice. Ultimately, these educational efforts aim to bridge the gap between clinical research and daily patient care, improving outcomes for diabetic individuals worldwide..

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

In conclusion, this clinical study confirms that complete blood count-derived inflammatory ratios—specifically the Platelet-to-Lymphocyte Ratio (PLR), Eosinophil-to-Lymphocyte Ratio (ELR), and Monocyte-to-Lymphocyte Ratio (MLR)—serve as valuable, accessible biomarkers for systemic low-grade inflammation in type

2 diabetes mellitus patients. The statistical analysis demonstrated highly significant differences in these cellular markers across distinct patient groups, highlighting their sensitivity to secondary microvascular and macrovascular complications. PLR increased progressively across cohorts, peaking within the diabetic nephropathy group (449.52

± 107 , $p = 0.0004^{**}$), which underscores its strong association with microvascular tissue degradation and thrombo-inflammatory pathways. Conversely, MLR reached its maximum value within the cardiovascular complications cohort (1.01 , $p = 0.0034^{**}$), confirming its utility as a sensitive indicator of monocyte-driven macrovascular plaque pathology. ELR values doubled in both complication groups compared to uncomplicated controls ($p = 0.0454^{**}$), demonstrating a generalized involvement of eosinophilic tissue modulation during advanced stages of metabolic disease. Importantly, these significant shifts occurred independently of patient age (p

$= 0.799$), which supports the diagnostic reliability of these ratios across varying adult demographics. Because these biomarkers are derived from routine blood counts, they offer an exceptional combination of clinical accuracy, cost-effectiveness, and universal availability. Integrating ELR, PLR, and MLR evaluations into routine clinical practice

could significantly improve early risk stratification, allowing for timely interventions that reduce morbidity and improve long-term outcomes for diabetic patients worldwide.

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**IMPLEMENTATION OF AGREEMENT
JOINT RESEARCH PROGRAM**



BETWEEN

Institut Ilmu Kesehatan Bhakti Wiyata Kediri AND

MAHSA University

No. 216/IK/E.2342/III-93/2025

This Arrangement sets forth Joint Research Agreement is made on this day by and between (Institut Ilmu Kesehatan Bhakti Wiyata Kediri) and (MAHSA University), hereinafter referred to singularly as “the Party” and collectively as “the Parties”.

WHEREAS, the Parties have decided to conduct a Joint Research Program on **Exploring the diagnostic value of the Eosinophil-Lymphocyte Ratio (ELR), Platelet-Lymphocyte Ratio (PLR) and Lymphocyte Ratio (MLR), in diabetes mellitus with cardiovascular and nephropathy complications as new biomarkers of low-grade inflammation**. NOW, therefore, in consideration of the mutual promises recited herein, the parties agree as follows:

I. Period of Services

(Fathul Hidayatul Hasanah) from Institut Ilmu Kesehatan Bhakti Wiyata Kediri and (Ikram Shah bin Ismail) from MAHSA University agree to conduct a joint research program on **Exploring the diagnostic value of the Eosinophil-Lymphocyte Ratio (ELR), Platelet-Lymphocyte Ratio (PLR) and Lymphocyte Ratio (MLR), in diabetes mellitus with cardiovascular and nephropathy complications as new biomarkers of low-grade inflammation** for the period of 2024/2025

II. Employee Status

During the term of this agreement, Fery Eko Pujiono will remain at all times an employee of Institut Ilmu Kesehatan Bhakti Wiyata. Institut Ilmu Kesehatan Bhakti Wiyata will be responsible for continuing and making all appropriate employee payrolls for Fery Eko Pujiono For no purpose will joint research program.

III. Funding

Each Party will each bear any costs, expenses, or other charges of whatever nature incurred by such Party and which are not expressly detailed in the approved Joint Research.

IV. JOINT INTELLECTUAL PROPERTY

- a) The protection of intellectual property rights shall be enforced in conformity with the respective national laws, rules and regulations of the Parties and with other international agreements signed by both Parties.
- b) The use of the name, logo and/or official emblem of any of the Parties on any publication, document and/or paper is prohibited without the prior written approval of either Party.
- c) The ownership of all intellectual property arising of the work or any project under this Agreement (hereinafter referred to as “Joint Intellectual Property”) shall be shared equally between both Parties.
- d) Either Party may, by giving a prior written notice to the other Party and upon certain payment, the amount of which to be agreed later by the Parties, to the other Party abandon its own share in the Joint Intellectual Property and transfer its share in the Joint Intellectual Property to the other Party.

V. PUBLICATIONS POLICY

Both Parties shall have the right to use and publish any information derived from the work or project under this Agreement provided that written consent is obtained from the other Party. In the event that any Parties, wishes to publish, disclose and/ or present (in any form of disclosure) the data and/ or outcome arising from this Agreement, the Party shall submit a draft of each such publication or presentation to the other Party and give the right to the other Party to have certain parts of the said publication. In any such publications, the contribution of both Parties shall be acknowledged.

VI. Duration of Agreement

This Agreement is valid for a year and becomes effective on the date that it is signed by the official representatives of both institutions. This Agreement may be terminated according to the same terms set forth in the Joint Research Agreement between Institut Ilmu Kesehatan Bhakti Wiyata Kediri and MAHSA University If either institution intends to terminate or to modify this Agreement, a written notice should be given to the other institution 30 days prior to the desired effective date of termination.

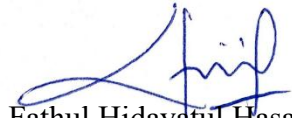
VII. Force Majeure

If, as a result of an act of force majeure, including without limitation, an act of God, war, riot, labor dispute, strike or threat thereof, intervention of a government agency or instrumentality or other occurrence beyond the control of either party, either party is substantially hindered in performing its obligations hereunder then, in such event, that party shall have the right, upon notifying the other of the occurrence of force majeure as herein defined, to suspend performance of the cooperative activity pursuant to this Agreement and any contract based on this Agreement until the force majeure has passed

IN WITNESS WHEREOF, the parties hereto have executed this agreement as of the date fit written above.

Signed by

Chief Researcher



Fathul Hidayatul Hasanah

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