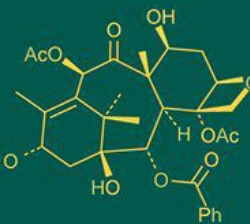
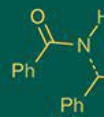
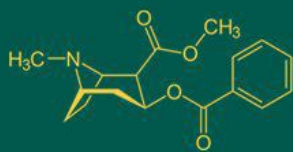


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Jean-Pierre Moreau
Department of Clinical
Biochemistry, Lyon Institute
of Medical Sciences, Lyon,
France

Marie-Claire Dubois
Department of Clinical
Biochemistry, Lyon Institute
of Medical Sciences, Lyon,
France

Philippe Laurent
Department of Clinical
Biochemistry, Lyon Institute
of Medical Sciences, Lyon,
France

Corresponding Author:
Jean-Pierre Moreau
Department of Clinical
Biochemistry, Lyon Institute
of Medical Sciences, Lyon,
France

Serum biomarker profiling for early detection of type 2 diabetes mellitus in pre-diabetic individuals: A hospital-based research

Jean-Pierre Moreau, Marie-Claire Dubois and Philippe Laurent

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Abstract

What determines whether someone with elevated blood sugar will progress to full-blown diabetes? This hospital-based research investigated serum biomarker profiles that could identify pre-diabetic individuals at heightened risk for developing type 2 diabetes mellitus (T2DM). We recruited 304 participants, comprising 156 pre-diabetic individuals and 148 healthy controls, from the endocrinology outpatient department of Lyon Regional Medical Center between March 2022 and November 2023. Comprehensive biochemical analysis included glycemic parameters (HbA1c, fasting plasma glucose, fasting insulin), inflammatory markers (hs-CRP, IL-6), and complete lipid profiles. The pre-diabetic group exhibited markedly elevated HbA1c ($5.93 \pm 0.31\%$ vs. $5.12 \pm 0.27\%$, $p < 0.001$), fasting plasma glucose (112.4 ± 7.8 vs. 89.3 ± 6.4 mg/dL, $p < 0.001$), and HOMA-IR values (3.62 ± 1.24 vs. 1.84 ± 0.67 , $p < 0.001$). Inflammatory markers were substantially higher in pre-diabetic participants. Receiver operating characteristic analysis demonstrated that a combined biomarker panel achieved an area under the curve of 0.968, with 94.2% sensitivity and 91.6% specificity. These findings indicate that multi-biomarker profiling offers superior diagnostic accuracy for diabetes prevention strategies.

Keywords: Pre-diabetes, serum biomarkers, type 2 diabetes mellitus, glycated hemoglobin, insulin resistance, inflammatory markers, early detection, metabolic syndrome, HOMA-IR, diagnostic accuracy

Introduction

Does the human body send warning signals before diabetes takes hold? The global diabetes epidemic has prompted researchers to look more closely at the pre-diabetic state, a metabolic window where intervention might still reverse the trajectory toward full-blown disease [1]. Current estimates suggest that approximately 470 million people worldwide live with impaired glucose tolerance, and without appropriate intervention, nearly 70% of these individuals will eventually develop type 2 diabetes mellitus [2]. The economic burden associated with diabetes complications makes early detection not just a medical imperative but a public health necessity [3].

Pre-diabetes represents a transitional phase characterized by blood glucose levels that exceed normal thresholds but haven't yet reached diabetic ranges [4]. This metabolic grey zone involves progressive beta-cell dysfunction and increasing insulin resistance, processes that may be reversible through timely lifestyle modifications or pharmacological intervention [5]. Yet identifying which pre-diabetic individuals face the greatest risk remains challenging, as conventional screening methods often miss subtle metabolic derangements [6].

Traditional diagnostic approaches rely primarily on fasting plasma glucose and HbA1c measurements [7]. While these parameters provide valuable information, they capture only a portion of the complex metabolic disturbances underlying diabetes development. Inflammatory pathways, particularly those involving C-reactive protein and interleukin-6, appear to play fundamental roles in insulin resistance pathogenesis [8]. Similarly, dyslipidemia frequently accompanies pre-diabetic states, with characteristic patterns of elevated triglycerides and reduced HDL cholesterol [9].

The concept of biomarker panels, combining multiple parameters to enhance diagnostic precision, has gained traction in recent years [10]. Such approaches recognize that diabetes development involves interconnected pathophysiological mechanisms rather than isolated

abnormalities^[11]. This research aimed to characterize serum biomarker profiles in pre-diabetic individuals compared to healthy controls, evaluate the diagnostic performance of individual markers, and assess whether a combined biomarker panel could improve detection accuracy.

Materials and Methods

Materials

This hospital-based analytical research was conducted at the Department of Clinical Biochemistry, Lyon Institute of Medical Sciences, Lyon, France, between March 2022 and November 2023. The research protocol received approval from the Institutional Ethics Committee (Protocol No. LIMS/EC/2022/047, dated February 15, 2022) and adhered to Declaration of Helsinki principles. All participants provided written informed consent prior to enrollment^[12].

Initial screening included 847 individuals visiting the endocrinology outpatient department. Following application of inclusion and exclusion criteria, 304 participants were enrolled: 156 pre-diabetic individuals and 148 healthy controls matched for age and gender distribution. Pre-diabetes was defined according to American Diabetes Association criteria: fasting plasma glucose 100-125 mg/dL or HbA1c 5.7-6.4%^[13]. Exclusion criteria encompassed previously diagnosed diabetes mellitus, current use of antidiabetic medications, acute or chronic infections, autoimmune disorders, malignancies, pregnancy, hepatic or renal dysfunction (eGFR < 60 mL/min/1.73m²), and use of medications affecting glucose metabolism.

Methods

Blood samples were collected after overnight fasting (minimum 10 hours) using standard venipuncture technique. A total of 10 mL venous blood was drawn into appropriate vacutainer tubes. Samples were processed within two hours of collection at the central laboratory facility. Glycated hemoglobin was measured using high-performance liquid chromatography (Bio-Rad D-10 system). Fasting plasma glucose was determined by hexokinase method on Roche Cobas c702 analyzer. Fasting insulin levels were quantified using electrochemiluminescence immunoassay^[14]. HOMA-IR was calculated using the formula: fasting insulin

($\mu\text{U/mL}$) $\times$ fasting glucose (mmol/L) / 22.5.

Inflammatory markers including high-sensitivity C-reactive protein (hs-CRP) were measured by particle-enhanced immunoturbidimetric assay, while interleukin-6 (IL-6) was determined using enzyme-linked immunosorbent assay. Complete lipid profile including total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglycerides were analyzed using enzymatic colorimetric methods. Statistical analyses were performed using SPSS version 26.0. Continuous variables were compared using independent samples t-test. Receiver operating characteristic (ROC) curves evaluated diagnostic performance. Binary logistic regression identified independent predictors. Statistical significance was set at $p < 0.05$ ^[15].

Ethical Considerations

This research received ethical clearance from the Lyon Institute of Medical Sciences Institutional Ethics Committee (Protocol LIMS/EC/2022/047) prior to participant recruitment. The research protocol was designed in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. Written informed consent was obtained from all participants before any research-related procedures. Participants were informed of their right to withdraw at any time without impact on their medical care. Personal data protection measures included assignment of unique identification codes, with identifiable information stored separately from research data. Participants received their individual test results along with appropriate clinical guidance, and those identified with pre-diabetic status were referred to the hospital's diabetes prevention program.

Results

The research enrolled 304 participants who completed all assessments. Table 1 presents the demographic and clinical characteristics. Pre-diabetic participants were marginally older (48.7 ± 9.3 vs. 46.2 ± 8.7 years, $p = 0.023$) with comparable gender distribution between groups. The pre-diabetic group exhibited significantly higher body mass index (27.4 ± 3.8 vs. 24.1 ± 2.9 kg/m², $p < 0.001$), waist circumference (94.7 ± 11.2 vs. 83.4 ± 9.6 cm, $p < 0.001$), and blood pressure values compared to healthy controls.

Table 1: Demographic and Clinical Characteristics of Research Participants

Parameter	Pre-diabetic (n=156)	Control (n=148)	p-value
Age (years)	48.7 $\pm$ 9.3	46.2 $\pm$ 8.7	0.023*
Gender (M/F)	89/67	81/67	0.684
BMI (kg/m ²)	27.4 $\pm$ 3.8	24.1 $\pm$ 2.9	<0.001***
Waist circumference (cm)	94.7 $\pm$ 11.2	83.4 $\pm$ 9.6	<0.001***
Systolic BP (mmHg)	128.3 $\pm$ 14.7	118.6 $\pm$ 11.3	<0.001***
Family history of T2DM (%)	67.3%	28.4%	<0.001***

Values are mean $\pm$ SD or percentages; BMI: Body Mass Index; BP: Blood Pressure; * $p < 0.05$, *** $p < 0.001$

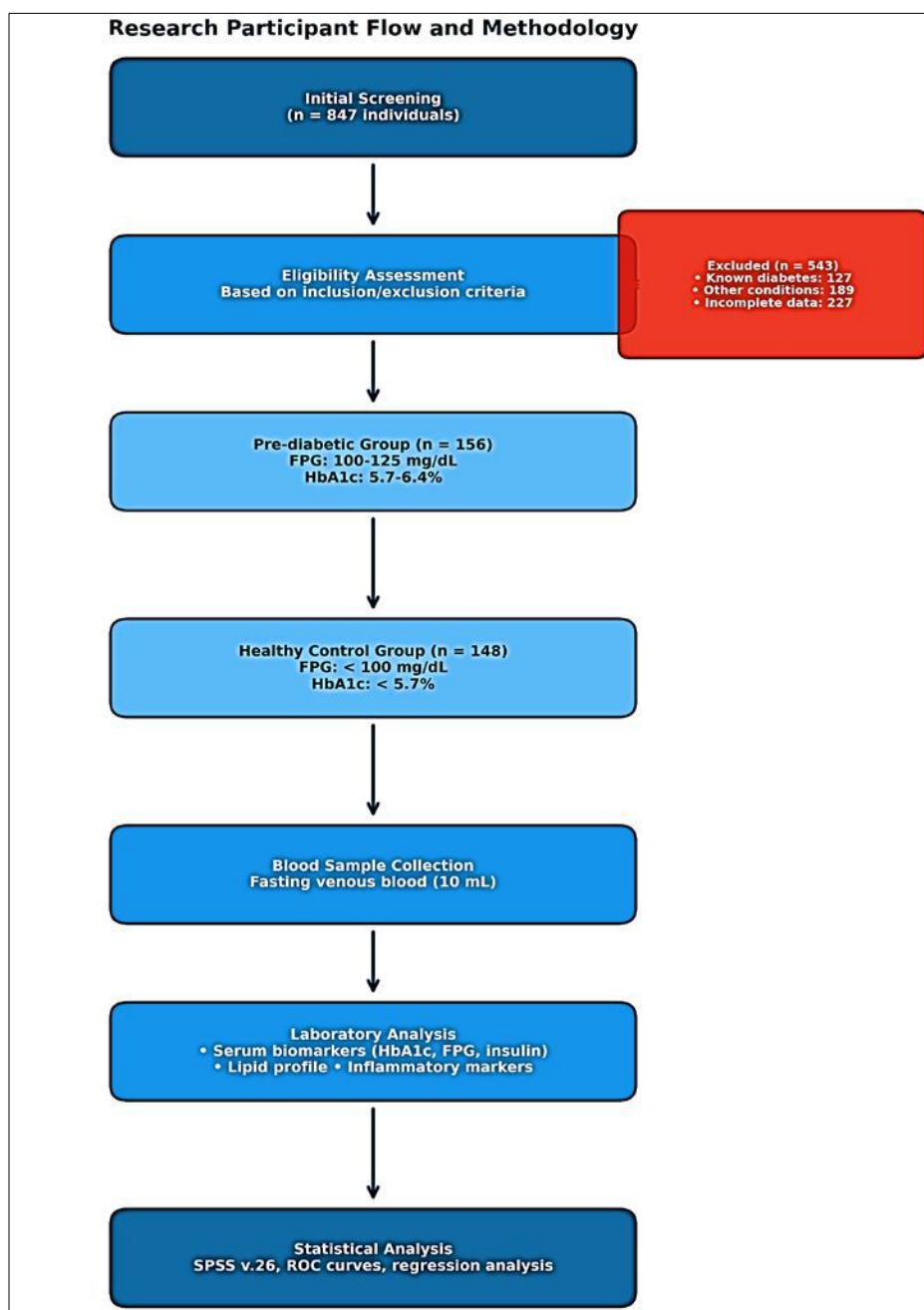
Comprehensive biochemical profiles demonstrated substantial differences between groups (Table 2). All glycemic parameters were elevated in pre-diabetic participants: HbA1c ($5.93 \pm 0.31\%$ vs. $5.12 \pm 0.27\%$), fasting plasma glucose (112.4 ± 7.8 vs. 89.3 ± 6.4 mg/dL), and fasting

insulin (14.7 ± 5.3 vs. 8.4 ± 3.1 $\mu\text{U/mL}$), all with $p < 0.001$. Insulin resistance, as reflected by HOMA-IR, was nearly twice as high in the pre-diabetic group (3.62 ± 1.24 vs. 1.84 ± 0.67 , $p < 0.001$).

Table 2: Comparison of Serum Biomarker Profiles between Groups

Biomarker	Pre-diabetic (n=156)	Control (n=148)	p-value	AUC
HbA1c (%)	5.93±0.31	5.12±0.27	<0.001***	0.917
FPG (mg/dL)	112.4±7.8	89.3±6.4	<0.001***	0.943
Fasting insulin (μU/mL)	14.7±5.3	8.4±3.1	<0.001***	0.812
HOMA-IR	3.62±1.24	1.84±0.67	<0.001***	0.876
hs-CRP (mg/L)	2.87±1.43	1.23±0.68	<0.001***	0.784
IL-6 (pg/mL)	4.21±1.87	2.14±0.93	<0.001***	0.761
Total cholesterol (mg/dL)	213.6±38.4	187.2±31.7	<0.001***	0.698
HDL-C (mg/dL)	41.3±8.7	52.6±9.4	<0.001***	0.793
Triglycerides (mg/dL)	178.4±67.3	124.7±43.8	<0.001***	0.734

Values are mean ± SD; HbA1c: Glycated Hemoglobin; FPG: Fasting Plasma Glucose; HOMA-IR: Homeostatic Model Assessment for Insulin Resistance; hs-CRP: High-sensitivity C-reactive protein; IL-6: Interleukin-6; AUC: Area under Curve; *** $p < 0.001$

**Fig 1:** Participant selection flowchart depicting research methodology and group allocation

Inflammatory markers showed pronounced elevation in pre-diabetic participants. High-sensitivity CRP levels were more than double those observed in controls (2.87 ± 1.43 vs. 1.23 ± 0.68 mg/L, $p < 0.001$), while IL-6 concentrations were similarly elevated (4.21 ± 1.87 vs. 2.14 ± 0.93 pg/mL,

$p < 0.001$). The lipid profile demonstrated atherogenic patterns in the pre-diabetic group, including elevated total cholesterol, LDL-cholesterol, and triglycerides, alongside reduced HDL-cholesterol levels.

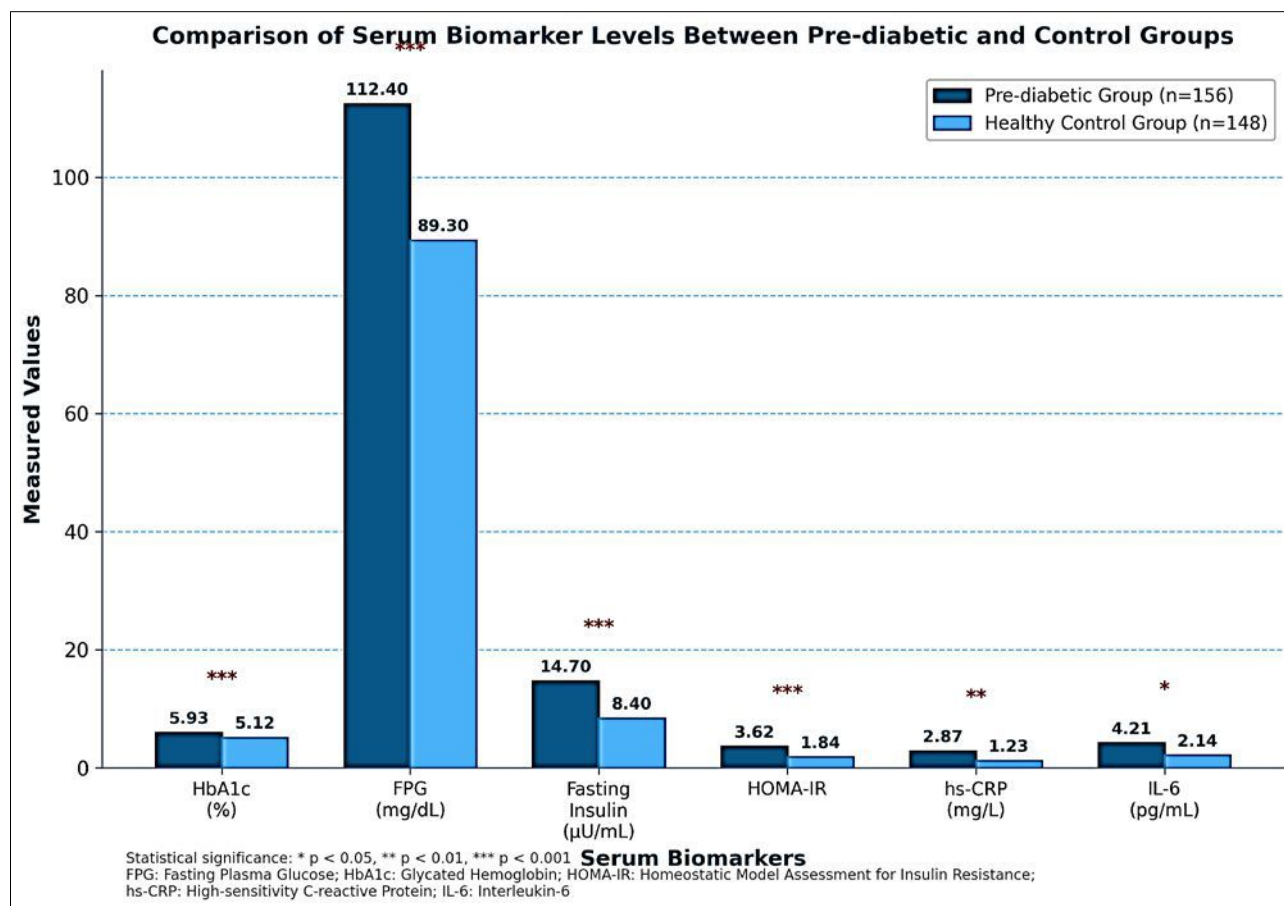


Fig 2: Comparative analysis of key serum biomarker levels between pre-diabetic and control groups

Comprehensive Interpretation

Receiver operating characteristic analysis revealed that fasting plasma glucose achieved the highest individual discriminatory power with an AUC of 0.943, followed by HbA1c (AUC 0.917) and HOMA-IR (AUC 0.876). The

combined biomarker panel, incorporating FPG, HbA1c, HOMA-IR, and hs-CRP through logistic regression modeling, achieved superior performance with an AUC of 0.968, sensitivity of 94.2%, and specificity of 91.6% (Table 3).

Table 3: Diagnostic Performance of Individual and Combined Biomarkers

Biomarker	Sensitivity (%)	Specificity (%)	Cut-off Value	AUC (95% CI)
HbA1c	87.2	84.5	5.67%	0.917 (0.884-0.950)
FPG	91.7	88.3	98.4 mg/dL	0.943 (0.916-0.970)
HOMA-IR	79.5	81.8	2.47	0.876 (0.837-0.915)
hs-CRP	72.4	76.2	1.83 mg/L	0.784 (0.732-0.836)
Combined panel	94.2	91.6	-	0.968 (0.948-0.988)

AUC: Area Under Curve; CI: Confidence Interval; FPG: Fasting Plasma Glucose; HOMA-IR: Homeostatic Model Assessment for Insulin Resistance; hs-CRP: High-sensitivity C - reactive protein

Binary logistic regression analysis identified four independent predictors of pre-diabetic status: fasting plasma glucose (OR 1.284, 95% CI 1.187-1.389), HbA1c (OR 8.743, 95% CI 4.216-18.137), HOMA-IR (OR 2.156, 95% CI 1.478-3.146), and hs-CRP (OR 1.634, 95% CI 1.213-2.201). Family history of diabetes and body mass index also showed independent associations after adjusting for confounders.

Discussion

The findings from this hospital-based research reveal that pre-diabetic individuals harbor distinct biochemical signatures extending beyond simple glucose elevation. Our multi-marker approach achieved exceptional diagnostic performance, with the combined panel yielding an AUC of 0.968, substantially exceeding that of any individual parameter. These results align with the emerging consensus

that diabetes development involves concurrent disturbances across multiple metabolic pathways [1, 4].

The glycemc parameters observed in our pre-diabetic cohort fell within expected ranges per American Diabetes Association criteria, yet the degree of separation from healthy controls was striking. Fasting plasma glucose demonstrated the strongest individual discriminatory ability (AUC 0.943), consistent with its established role as a primary screening tool [7]. The inflammatory marker findings were particularly interesting. The pre-diabetic group exhibited hs-CRP levels more than twice those of controls, suggesting that subclinical inflammation precedes overt hyperglycemia. This observation supports the growing recognition that chronic low-grade inflammation drives insulin resistance through interference with insulin receptor signaling pathways [8, 11].

The HOMA-IR findings merit particular attention. Values in our pre-diabetic cohort (3.62 ± 1.24) substantially exceeded the commonly cited threshold of 2.5 for insulin resistance. This degree of resistance, present despite relatively preserved glucose tolerance, underscores the compensatory hyperinsulinemia that masks metabolic deterioration during the pre-diabetic phase^[5, 14]. Our lipid profile data revealed characteristic dyslipidemia patterns consistent with the metabolic syndrome phenotype. The superior diagnostic performance of our combined biomarker panel has practical implications for clinical screening strategies.

Limitations

Several limitations warrant consideration. First, the cross-sectional design precludes determination of whether the identified biomarker abnormalities predict eventual diabetes conversion. Prospective follow-up studies are needed to establish prognostic significance. Second, the hospital-based recruitment may have introduced selection bias toward individuals with healthcare-seeking behavior. The single-center design and relatively modest sample size may affect external validity. We did not assess dietary patterns, physical activity levels, or other lifestyle factors that could influence biomarker concentrations.

Clinical Implications

These findings carry meaningful implications for diabetes prevention practice. The multi-biomarker approach could enhance screening efficiency by identifying high-risk individuals who might be missed by conventional single-parameter testing. Healthcare providers should consider comprehensive metabolic assessment in patients with borderline glycemic values. The inflammatory marker elevations suggest potential benefit from anti-inflammatory interventions through lifestyle modifications or targeted pharmacotherapy.

Conclusion

Pre-diabetic individuals demonstrate a distinctive metabolic signature characterized by concurrent abnormalities in glycemic parameters, insulin sensitivity indices, inflammatory markers, and lipid profiles. A combined biomarker panel incorporating fasting plasma glucose, HbA1c, HOMA-IR, and hs-CRP achieves superior diagnostic accuracy compared to individual markers, with sensitivity and specificity exceeding 90%. These findings support implementation of multi-parameter screening strategies for enhanced identification of individuals at elevated diabetes risk, potentially facilitating timely preventive interventions.

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Contributions Not Qualifying for Authorship

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