Investigation of the AgNOR (Argyrophilic Nucleolar Organizing Region) Protein Levels in Patients with Coronary Artery Diseases

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ABSTRACT

Objective: This study aimed to evaluate the prognostic significance of Argyrophilic Nucleolar Organizing Region (AgNOR) proteins in patients with acute myocardial infarction (AMI) and to determine their potential role in predicting the extent of myocardial damage.

Materials and Methods: A case-control study was conducted with 20 AMI patients and 17 healthy controls. Peripheral blood samples were stained to assess AgNOR protein levels. The AgNOR parameters, such as the number of AgNORs and the total AgNOR area to total nuclear area (TAA/NA) ratio, were analyzed using ImageJ software. Statistical analyses were performed using SPSS to assess differences between groups and correlations with clinical markers.

Results: The study revealed a significant increase in both the mean AgNOR number and TAA/NA ratio among AMI patients compared to controls (p < 0.01). These parameters also correlated with known cardiac damage markers such as Troponin I level. Sensitivity (100%) and specificity (100%) analysis indicated that these AgNOR parameters could effectively differentiate between AMI patients and healthy individuals.

Conclusion: AgNOR proteins emerge as a promising and dependable biomarker for evaluating myocardial damage and predicting patient prognosis in cases of AMI. Their remarkable sensitivity and specificity in distinguishing AMI cases underscore their potential clinical utility. However, further studies with larger cohorts are imperative to validate these findings.

Keywords: Acute Myocardial Infarction, AgNOR Proteins, Prognostic Biomarkers, Cardiac Damage, Troponin.

INTRODUCTION

The precipitous onset of acute myocardial infarction (AMI), a pivotal medical emergency, often heralds the culmination of underlying coronary artery pathology. The intricate interplay between atherosclerotic progression and immune response elements is crucial, as cellular constituents of the immune system congregate within arterial plaques, exacerbating the inflammatory milieu and furthering the atherosclerotic process (1, 2). In the throes of AMI, a critical infiltration of immune cells such as neutrophils, macrophages, and lymphocytes into the ischemic myocardial tissue occurs, particularly within the initial quintet of hours post-reperfusion. Among these, T lymphocytes assume a central role in orchestrating the inflammatory cascade by stimulating various cell types through cytokine secretion, notably interferon-gamma, shaping the pathophysiological landscape of AMI.

The nucleolar organizer regions (NORs), composed of ribosomal DNA and associated proteins, some of which demonstrate argyrophilic properties, serve as the architectural framework for ribosomal gene transcription. Upon silver staining, these regions manifest as discernible blackened domains within the nucleolar confines, known as AgNORs. AgNOR proteins have been the focal point of research across a spectrum of cellular environments, including but not limited to hair roots, epithelial linings, and pulmonary and muscular tissues. Notably, these studies have unveiled a rise in AgNOR protein levels in response to hypoxic stress, suggesting a putative protective mechanism.
In the context of AMI, myocardial ischemia is a direct consequence of abrupt coronary thrombosis. Despite the critical nature of this event, the literature is bereft of investigations into the role of AgNOR proteins in the setting of AMI. This present study is pioneering in evaluating AgNOR protein levels in patients with ST-elevation myocardial infarction (STEMI), setting a comparative stage with individuals devoid of acute coronary syndromes.

Further, AgNOR staining has emerged as a formidable technique for nucleolar visualization in interphase nuclei, grounded in the transcriptional activity of ribosomal RNA genes within the nucleolus. Given the ubiquity of ribosomal DNA gene clusters across human acrocentric chromosomes, this method bears significance for various applications of chronic diseases (3), extending from tumor pathology to developmental studies and beyond (4, 5).

The nexus between AgNOR proteins and myocardial ischemia remains uncharted in cardiological research (6). Consequently, this prospective study seeks to elucidate the influence of acute cardiac ischemia on NOR protein synthesis within cardiac cells and to discern any associative patterns between AgNOR protein abundance and myocardial tissue damage. Our research aims to establish AgNOR proteins' sensitivity to cellular metabolic perturbations as a morphological biomarker, with the ultimate goal of improving the management and prognosis of AMI.

MATERIAL and METHODS

Study Population: This case–control study was conducted with a cohort of 20 patients diagnosed with acute myocardial infarction (AMI) aged between 42 to 85 years admitted to the Emergency Department (ED), with a clinical exacerbation between October 2016 and March 2017 (Group 1). A control group (Group 2) consisted of 17 age-matched healthy individuals ranging from 38 to 88 years, presenting to the emergency service without any signs of tissue ischemia or hypoxia. Patients with known inflammatory comorbidities or chronic diseases (3), extending from tumor pathology to age. Correlations between clinical data and AgNOR parameters were assessed using Pearson or Spearman correlation analysis, based on the data distribution. A p-value of less than 0.05 was considered statistically significant.

RESULTs

In our investigation involving a cohort of 20 acute myocardial infarction (AMI) patients (average age 69.20 ± 9.92 years) and 17 healthy controls (average age 69.52 ± 16.57 years), we found no significant age differences between the groups. The demographic characteristics are detailed in Table 1.

The lymphocyte nucleoplasm of both AMI patients and healthy controls were subjected to silver staining to assess AgNOR parameters. AMI patients demonstrated a notably higher mean AgNOR number and TAA/NA ratio than the controls (P < 0.01 for both), as shown in Table 2.

Among the AMI group, 45% required hospitalization, yet there was no significant disparity in mean AgNOR numbers or TAA/NA ratios when comparing those hospitalised to those not. However, hospitalized AMI patients exhibited significantly elevated pCO2 levels (P = 0.003). These findings are summarized in Table 3.

Histological analysis revealed substantial variances in the AgNOR-stained lymphocyte nucleoplasm between AMI patients and healthy controls. AMI patients' lymphocytes were typically irregular in shape with abundant silver-stained dots, as illustrated in Figure 1a–b.
The sensitivity and specificity analyses of the AgNOR parameters showed that the TAA/TNA ratio and the mean AgNOR number had 100% sensitivity and specificity in differentiating AMI patients from healthy controls. However, these parameters were less sensitive and specific in predicting the need for hospitalization post-AMI, as depicted in Figure 2.

There was a strong positive correlation between troponin T levels and hospitalization necessity (r = 0.845; P < 0.001). A significant positive correlation was also observed between Troponin T levels and the mean AgNOR number (r = 0.624; P = 0.03).

### Table 1. Demographic data of the patients with acute myocardial infarction (AMI) and healthy control subjects.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients with AMI (n = 20)</th>
<th>Healthy control subjects (n = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>69.20 ± 9.92</td>
<td>69.52 ± 16.57</td>
</tr>
<tr>
<td>Age range, years</td>
<td>42-85</td>
<td>38-88</td>
</tr>
<tr>
<td>Sex, male/female</td>
<td>14/6</td>
<td>11/6</td>
</tr>
</tbody>
</table>

Data presented as mean ± SD or n of patients. No statistically significant between-group differences (P ≥ 0.05).

### Table 2. Argyrophilic nucleolar organizing region-associated protein (AgNOR) parameters for patients with acute myocardial infarction (AMI) and healthy control subjects.

<table>
<thead>
<tr>
<th>AgNOR parameter</th>
<th>Patients with AMI (n = 20)</th>
<th>Healthy control subjects (n = 17)</th>
<th>Z value</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAA/TNA ratio, %</td>
<td>0.540 ± 0.014</td>
<td>0.187 ± 0.044</td>
<td>–4.243</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>AgNOR number</td>
<td>4.038 ± 0.644</td>
<td>1.662 ± 0.208</td>
<td>–4.243</td>
<td>P &lt; 0.01</td>
</tr>
</tbody>
</table>

Data presented as mean ± SD. Groups compared using Mann–Whitney U–test. TAA/TNA, total AgNOR area/total nuclear area.

### Table 3. Argyrophilic nucleolar organizing region-associated protein (AgNOR) parameters and Troponin T value for patients with acute myocardial infarction (AMI) stratified according to their severity.

<table>
<thead>
<tr>
<th></th>
<th>Cardiac arrest patients after AMI (n = 9)</th>
<th>Non-arrest patients with AMI (n = 11)</th>
<th>Z Value</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Troponin T(min–max) ng/mL</td>
<td>10.98 ± 16.9 (3.75–57.2)</td>
<td>0.001 ± 0.4 (0.001–1.02)</td>
<td>–3.007</td>
<td>P = 0.003</td>
</tr>
<tr>
<td>TAA/TNA, %</td>
<td>0.243 ± 0.041</td>
<td>0.181 ± 0.047</td>
<td>–0.266</td>
<td>NS</td>
</tr>
<tr>
<td>AgNOR number</td>
<td>3.847 ± 0.521</td>
<td>4.153 ± 0.601</td>
<td>–1.178</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data presented as mean ± SD and min–max. Groups compared using Mann–Whitney U–test. pCO2, partial pressure of carbon dioxide; TAA/TNA, total AgNOR area/total nuclear area; NS, no statistically significant between-group difference (P ≥ 0.05).

**Figure 1:** Morphological Analysis of AgNOR Proteins in Acute Myocardial Infarction Nuclei. **A.** Demonstrated are variably sized AgNOR dots within nuclei from patients experiencing AMI, characterized by irregular shapes. **B.** Depicted is the aggregation of irregular AGNOR staining, indicative of protein levels within affected tissues. Magnification: ×1000, oil immersion technique. Scale bar represents 50 µm.
This study aimed to investigate the prognostic potential of AgNOR (Argyrophilic Nucleolar Organizing Region) protein levels in patients with acute myocardial infarction (AMI). By comparing AgNOR parameters between AMI patients and healthy controls, our research provides compelling evidence that heightened AgNOR protein levels are significantly associated with the extent of myocardial damage in AMI patients. Our study demonstrated a substantial elevation in the mean number of AgNORs and TAA/TNA (total AgNOR area/total nuclear area) ratios in patients with ST-elevation myocardial infarction (STEMI) compared to a healthy control group, echoing previous findings that link AgNOR protein levels to cellular hypoxic stress responses (3, 5, 10).

Notably, this aligns with earlier research suggesting that AgNOR protein proliferation may serve as a protective reaction by immune system cells early in the MI process. The methodology employed—AgNOR staining of lymphocyte nucleoplasm—offers a robust and quantifiable means of assessing cellular response to myocardial injury. The sample size, while modest, was adequate to demonstrate clear statistical significance in AgNOR protein levels between the study groups. Despite the inherent limitations of a single-center study, the methodological rigor and adherence to ethical standards lend credibility to the findings.

Our results resonate with prior research that linked increased AgNOR protein levels to hypoxic conditions in different cellular environments (3, 11, 12). Our study's significant correlation between AgNOR parameters and cardiac damage markers is consistent with literature suggesting the prognostic utility of nucleolar organizer regions in various pathologies (13, 14). However, our study's novelty lies in applying these parameters to AMI prognosis.

We observed that AgNOR parameters differentiated AMI patients from healthy controls with high sensitivity and specificity and correlated with clinical markers of myocardial injury severity, such as troponin levels. This correlation with troponin, a well-established marker of myocardial damage, underscores the potential of AgNOR proteins to serve as a supplementary tool in clinical settings. Furthermore, the positive correlation with known cardiovascular risk factors suggests that AgNOR proteins may play a more extensive role in the pathophysiology of cardiovascular diseases than previously recognized.

The specificity and sensitivity of AgNOR parameters in distinguishing STEMI patients from the control group are particularly noteworthy, with the TAA/TNA ratio displaying 100% sensitivity and 100% specificity. Such high predictive values suggest that AgNOR quantification could be a significant adjunct to traditional markers like troponin levels, which we found strongly correlated with the necessity for hospitalization post-AMI.

In light of our findings, AgNOR proteins emerge as potential biomarkers for myocardial damage assessment. The implications of these findings could be profound, influencing the stratification of AMI patients for therapeutic interventions and potentially informing clinical decisions regarding prognosis. This is particularly salient for STEMI patients, for whom timely and accurate prognostic assessments are critical.
CONCLUSION

In conclusion, our study furnishes compelling evidence supporting the significant prognostic role of AgNOR proteins in acute myocardial infarction (AMI). The clear correlation observed between AgNOR protein levels and myocardial damage, alongside the high sensitivity and specificity of AgNOR parameters, suggests a promising future for these markers in clinical practice. Nevertheless, the establishment of AgNOR protein levels as a standard prognostic tool in AMI requires further research, including larger cohorts and multi-center trials. Future studies should delve into clarifying the molecular mechanisms underlying the relationship between AgNOR proteins and myocardial injury, potentially paving the way for novel therapeutic targets. Our research specifically indicates a significant correlation between AgNOR protein levels and the extent of cardiac damage in AMI patients. The quantification of AgNOR proteins could serve as a critical metric, offering insights into the severity of cardiac injury and potentially acting as a predictive biomarker for patient prognosis in AMI cases. Furthermore, the measurement of AgNOR parameters may emerge as an innovative method for anticipating mortality risk during AMI episodes. While our findings underscore the value of AgNOR parameters as reliable indicators in the context of ST-elevation myocardial infarction (STEMI), the establishment of these parameters as a standard prognostic tool will require validation through extensive longitudinal studies with a larger patient cohort.

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Conflict of interest: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author Contributions: The study was conceptualized and designed by F.T.S. and R.E. Data analysis was systematically conducted by F.T.S. and R.E., with the investigation being led by both authors. The methodological framework was developed collaboratively by F.T.S. and R.E., and visual data representation was prepared by the same team. The initial draft of the manuscript was authored by F.T.S., B.Y., and R.E., while subsequent revisions were carried out by F.T.S., B.Y., and R.E. All participating authors have thoroughly reviewed and consented to the final manuscript as published.

Ethical approval: The present study was conducted in strict accordance with the principles outlined in the Declaration of Helsinki. Ethical approval for the study was obtained from the appropriate ethics committee, and all participants provided informed consent before participating in the study. Study received approval from the Ethics Committee of Düzce University, designated by the ethical approval code: 2016/62.

REFERENCES


