Neutrophilic Ribonucleic Acid Expression as a Clinical Tool in Detecting Cerebral Aneurysms

Intracranial aneurysms (IAs) can have catastrophic consequences when they rupture. Despite their potentially fatal nature, nearly 6% of the American population unknowingly lives with IAs. The current gold standard for detecting IAs is digital subtraction angiography (DSA). An inexpensive and sensitive blood test screening method for IAs would prove useful given the cost ineffectiveness and risks of DSA. Previous studies have shown persistent inflammation with neutrophilic response in aneurysmal endothelium, making neutrophils a potential screening target for IAs. Given the inflammatory microenvironment of cerebral aneurysms and the previously found connection between RNA expression and vascular disease, Tutino et al. hypothesized that circulating neutrophils carry a unique transcriptional signature of IAs.

The authors first collected 77 peripheral blood samples from patients undergoing DSA, with 35 positive IA patients and 42 negative IA patients. Cerebral imaging was done to confirm the IA diagnosis, with confirmed IA positive patients matched to healthy controls. Patients with potentially inflammatory conditions such as pregnancy, recent invasive surgery, chemotherapy, severe pyrexia as well as other cerebrovascular malformations and abdominal aortic aneurysms were also excluded. Collected blood samples were centrifuged and neutrophil RNA was extracted using TRIzol (Life Technologies, Carlsbad, California). Prior to sequencing, IA patient samples were matched with controls based on demographics and comorbidities. RNA was sequenced at the authors’ university’s Next-Generation Sequencing and Expression Analysis Core facility, where gene expression levels were calculated. Differential gene expression analysis between IA and control groups was also performed to identify unique IA-associated signatures. Differences in expression levels were confirmed using quantitative reverse transcription polymerase chain reaction. Of the 77 collected blood samples, 37 were adequate for RNA sequencing (16 IA, 21 control). This was narrowed to 11 IA and 11 controls after experimental-control matching. About 13,377 transcripts had testable expression differences between the 2 groups, with 258 of those being significantly different (P < .05). The authors defined an IA-associated RNA expression difference as a change by a factor of 2 or more, thereby decreasing the 258 significant transcripts to 82 potential IA associated RNA signatures. Dimensionality reduction analysis using principal component analysis (PCA) and multidimensional scaling (MDS) was performed on all neutrophil transcriptome data to visually distinguish the degree to which RNA expression can differentiate IA and control groups.

Out of the 258 differentially expressed neutrophilic transcripts in the IA group, most were involved in defense response, leukocyte activation, cell activation, maintenance of cell number, stem cell activation, and stem cell development. Physiological pathway modeling was then utilized and showed network activation consistent with cellular movement and cardiovascular system function, cell-to-cell signaling, organismal injury, cell proliferation, and tissue morphology (Figure). These functions are consistent with neutrophilic response to cerebrovascular insult. Specifically, increased differential expression of transcripts in the IA group showed network activation of extracellular signal—regulated kinase1- and 2-(ERK1/2) and transcription factor activated protein-1 (AP-1). Finally, in a new, unpaired population consisting of 5 IA patients and 5 controls, the authors applied the newly discovered neutrophil RNA signatures to validate the accuracy with which patients can be assigned to a group without or with an intracranial aneurysm. A total of 9 out of 10 samples were accurately assigned according to PCA and hierarchical clustering. The authors acknowledged the small patient population and recommend replicative studies with larger sample sizes.

Ultimately, in this early work, the authors identified 82 unique neutrophilic RNA signatures associated with IAs. These signatures demonstrated efficacy in discerning IA patients from controls in a heterogeneous, although carefully-selected, population. Several issues remain that will have to be answered in the future. The sample size was very small putting into question whether the test could be applied to a larger population. Next, it is unclear how well the authors would be able to control for many inflammatory conditions present in the general patient population without the test generating false-positive results. Though not a perfect screening test, this preliminary study generated a novel method that appears...
FIGURE. Models based on differential expression of transcripts in intracranial aneurysm and control patient populations show network regulation by A, ERK1/2 and AP-1, B, ubiquitin-C, C, vascular endothelial growth factor, and D, interferon-gamma. Reprinted from Tutino et al.3 CC BY 4.0.

cost effective and noninvasive, suggesting a role for blood-based testing in detection of cerebral aneurysms.

Disclosure

The authors have no personal, financial, or institutional interest in any of the drugs, materials, or devices described in this article.

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