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Pediatric Issue

Moyamoya Biomarkers

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Moyamoya disease (MMD) is an arteriopathy of the intracranial circulation predominantly affecting the branches of the internal carotid arteries. Heterogeneity in presentation, progression and response to therapy has prompted intense study to improve the diagnosis and prognosis of this disease. Recent progress in the development of moyamoya-related biomarkers has stimulated marked interest in this field. Biomarkers can be defined as biologically derived agents–such as specific molecules or unique patterns on imaging–that can identify the presence of disease or help to predict its course. This article reviews the current categories of biomarkers relevant to MMD–including proteins, cells and genes–along with potential limitations and applications for their use.

Key Words : Moyamoya · Biomarker · Stroke · Genetics · Proteome.

INTRODUCTION

Moyamoya is a progressive arteriopathy of unclear etiology that predominantly affects the major branches of the internal carotid arteries[58,68]. First described in Japan, this disorder was initially characterized by radiographic criteria, as defined by Suzuki and Takaku[68]. These elegant anatomic studies have served as the foundation underlying decades of clinical and laboratory efforts to better understand this disease. Over time, it has increasingly become apparent that these angiographic findings represent a multitude of distinct pathophysiologic processes that manifest a shared radiographic signature[9,58].

Initially, this concept of multiple conditions culminating in a common end pathway was acknowledged by the distinction between moyamoya "disease" (bilateral arteriopathy existing in isolation) and moyamoya "syndrome" (either unilateral arteriopathy or arteriopathy found in conjunction with some other medical disorder)[9,58]. By convention, the arteriopathies are usually collectively referred to as "moyamoya disease" (MMD). This awareness of heterogeneity in clinical presentation has prompted research focused on the development of techniques capable of better defining MMD. One area with the potential to advance the diagnosis, prognosis and understanding of MMD is the field of biomarkers.

Biomarkers are defined as "any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease"[66]. In essence, biomarkers are biological "fingerprints" that can be used by clinicians to identify a specific disease. Broadly speaking, this definition can include physiological signs that are associated with a clinical phenotype, anatomic structures that can be identified radiographically as a distinct signature, molecular "fingerprints" of specific proteins measured in patient samples, unique cell types associated with disease or genetic mutations. This manuscript summarizes current biomarkers relevant to the diagnosis of MMD, including phenotypes, radiographic findings, proteins, circulating cells and genetic/epigenetic markers, followed by discussion of how they could be employed in practice.

PHENOTYPES

Moyamoya disease was first described in 1957 in Japan when the clinical symptoms of cerebral infarction were found in conjunction with "hypoplasia" of the internal carotid arteries[70]. Over the following 12 years, increasing recognition of common patient presentations allowed clinicians to couple specific symptoms with a distinct radiographic pattern, as ultimately reported by Suzuki[68]. While the need to rely solely on clinical observation to define phenotypes is being supplanted by imaging, molecular profiling and genetic analysis, awareness of associations between specific medical conditions and MMD is still important in practice.

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Although only loosely contained within the rubric of biomarkers, a list of medical conditions linked to MMD is useful. In addition to the practical application of helping physicians recognizing phenotypes that may be at-risk for arteriopathy, translational research efforts can be informed by clinical observation. Table 1 outlines some of the more common disorders that have been reported in conjunction with MMD (Table 1)[3,6,29,34,35,47,51,58,61,71].

RADIOGRAPHIC

Radiographic biomarkers have emerged as critical tools to identify disease and stratify risk in patients[64]. The initial description of MMD is predicated on a radiographic signature, the "puff of smoke" on catheter arteriography[68]. Since then, this radiographic biomarker has been used as the primary foundation upon which to build a definition of MMD[9,63]. Specific characteristics have been codified in order to assist clinical decision making, such as determining risk of stroke, chance of hemorrhage, likelihood of co-existing genetic conditions and need for surgery[9,11,14,25,26,30]. There is great variability in the current utilization of radiographic biomarkers across institutions that care for patients with MMD. However, a number of key markers have been cited repeatedly and have gained general acceptance. These signature findings are summarized in Table 2 and have been adopted in the International Classification of Diseases (Table 2)[9,58,63].

Radiographic biomarkers for MMD can be utilized in a wide array of clinical scenarios (Table 3). Unique patterns of vessel branching on arteriogram may indicate specific mutations, such as ACTA2 (discussed below)[48]. Distinct anomalies, such as posterior cerebral artery stenosis, may portend a higher risk of hemorrhage[14]. Any vessel anomaly identified by angiography on the non-affected hemisphere in unilateral MMD may increase the chance of subsequent progression to bilateral disease[28,62]. Changes in radiographic studies may predict response to therapy more accurately and can be used in formulating follow-up plans for patients[10,25,37,63]. In addition to functional perfusion studies, indirect measures of blood flow, such as reduction in the "ivy sign" on MRI may serve to mark a successful response to surgery[8,27]. Specific molecules can be detected with magnetic resonance spectroscopy and these metabolites may aid in prognosis[13]. More recently, imaging of patients pre- and post-operatively using positron emission tomography (PET) modified with novel labeled peptides has improved the potential to non-invasively observe the biological process of angiogenesis[5,33]. These findings underscore the immense potential of radiographic biomarkers to aid in the care of patients with MMD.

Table 1. Conditions reported in association with moyamoya syndrome

More common	Less common
Neurofibromatosis type I	Structural cardiac anomalies
Sickle cell disease	PHACEs syndrome
Down syndrome (trisomy 21)	Hyperthyroidism
Post-cranial radiation	Congenital dwarfing syndromes
	Alagille syndrome

PHACEs : posterior fossa malformations-hemangiomas-arterial anomalies-cardiac defects-eye abnormalities-sternal cleft and supraumbilical raphe syndrome

Table 2. Diagnostic radiographic biomarkers of moyamoya

Diagnostic radiographic biomarkers of moyamoya
Stenosis of the distal (intracranial) ICAs, up to and including the bifurcation, along with segments of the proximal ACA and MCA.
Dilated basal collateral vessels must be present (to varying degrees, depending on stage).
Findings must be bilateral (syndrome may be unilateral).

ICA : internal carotid artery, ACA : anterior cerebral artery, MCA : middle cerebral artery

PROTEINS

Measuring levels of proteins in body tissues or fluids as biomarkers requires that the proteins are somehow related to the presence of the disease, either directly or indirectly. It can sometimes be difficult to ascertain if the putative biomarker is produced directly from the pathologic tissue (such as prolactin from a prolactinoma) or as a secondary response of the body to the disease (such as C-reactive protein in the setting of a bacterial infection). Where one looks for a biomarker−tissue, spinal fluid, serum, urine, saliva−is usually secondary to discovering a clinically relevant molecule. Generally speaking, there are two major approaches to biomarker discovery; hypothesis driven discovery (in which specific molecules are selected a priori due to suspected roles in the given pathophysiologic processes) and proteomic screening (in which the entire proteome of specimens from diseased patients are compared to matched controls to reveal differences in expression).

Table 3. Radiographic biomarkers of moyamoya

Radiographic biomarker	Example of utilization
Anatomic	Demonstration of new vessels indicating surgical response (angiogram) Pattern recognition for genotype identification and risk assessment (angiogram for ACTA2 mutation, PCA anatomy as prognostic marker for stroke)
Functional	Indirect marker of ischemia (MRI ivy sign) Cerebrovascular reserve (SPECT with acetazolamide challenge, perfusion MRI)
Molecular	Direct detection of molecules non-invasively (MRS) Observation of biological processes (PET for angiogenesis)

PCA : posterior cerebral artery, SPECT : single-photon emission computerized tomography, MRS : magnetic resonance spectroscopy, PET : positron emission tomography

In looking for candidate biomarkers for MMD, both approaches have been applied. Initial efforts focused largely on the cerebrospinal fluid (CSF), given the proximity to the disease process in the cerebral vessels. General proteome analysis of the CSF has been applied by many groups around the world[2,43,56,65,69,75]. These studies have identified a number of expected candidate molecules related to ischemia and angiogenesis (as outlined below), but also some molecules of unclear pathophysiologic relevance, including oxyntomodulin, urocortin-2, beta-defensin 133, antibacterial protein LL-37 and liver-expressed antimicrobial peptide 2[2]. One of the most interesting recent applications of mass spectrometry in MMD biomarker discovery was the report of a novel peptide, 4473Da, that appeared to correlate with favorable postoperative collateral development in a small cohort of patients[43]. The significance of these findings continues to be assessed in studies with larger cohorts of patients.

Another method of organizing biomarker discovery is to categorize subgroups of molecules by function. Proteins linked to MMD include the broad classes of enzymes, growth factors, adhesion molecules and inflammatory/coagulation peptides (summarized in Table 4). These often result from hypothesis-driven biomarker discovery, but it can be difficult to ascertain if the molecules are uniquely causative of the disease or secondary by-products of the arteriopathy. While these markers have utility within the context of MMD, it will be important to direct future research toward determining which markers (if any) comprise a MMD-specific "fingerprint" and which might be more non-specific markers of general physiological processes like ischemia or angiogenesis.

Table 4. Molecular biomarkers of moyamoya

Classes of protein	Specific biomarkers	Level in moyamoya patients
Enzymes	Alpha 1 antitrypsin[1]	Increased
	MMP-3[23]	Decreased
	MMP-9[7,23]	Increased
	TIMP-1, -2[23]	Decreased
Growth factors	VEGF[23]	Increased
	PDGF BB[23]	Increased
	HGF[49]	Increased
	bFGF[42,65,69]	Increased
	TGF-beta[15]	Increased
Adhesion molecules	VCAM-1[65]	Increased
	ICAM-1[65]	Increased
	E-selectin[65]	Increased
	CRABP-1[32]	Increased
Inflammation/ coagulation	MCP-1[23]	Increased
	IL-1 beta[23]	Increased
	ANCA[73]	Increased
	D-dimer[4]	Increased
	SDF-1 alpha[50]	Increased

MMP : matrix metalloproteinase, TIMP : tissue inhibitor of metalloproteinase, VEGF : vascular endothelial growth factor, PDGF : platelet-derived growth factor, HGF : hepatocyte growth factor, bFGF : basic fibroblast growth factor, TGF-beta : transforming growth factor beta, VCAM-1 : vascular cell adhesion molecule 1, ICAM-1 : intercellular adhesion molecule 1, CRABP-1 : cellular retinoic acid binding protein 1, MCP-1 : monocyte chemotactic protein-1, IL-1 beta : interleukin 1 beta, ANCA : anti-neutrophil cytoplasmic antibodies, SDF-1 alpha : stromal cell-derived factor 1

CIRCULATING CELLS

Circulating cells have been employed as markers of disease in other fields, most notably in cancer research. Recently, these efforts have been expanded to include MMD. Central to the premise of this work is the hypothesis that MMD involves ongoing vascular remodeling, including both the primary arteriopathy and also the secondary angiogenesis from collateral development. Consequently, investigators have searched for endothelial cells and smooth muscle cells involved in these processes.

Pathological studies have long shown that proliferation of smooth muscle cells in the walls of the affected arteries in MMD is a common finding[44]. Smooth muscle progenitor cells (SPCs) have been isolated from the blood of patients with MMD. Recent analysis of these cells show that SPCs from moyamoya patients demonstrate irregular tube formation in assays when compared to SPCs from matched controls[24]. In addition, these SPCs exhibited differential expression of over 200 genes, including reduced CD31 expression, relative to controls from healthy individuals[24]. The ability to isolate a specific cell type and demonstrate differences in both protein expression and cell function suggest a dynamic approach to biomarker discovery in MMD.

Parallel to this work with smooth muscle cells, investigation into the role of endothelial cells as markers of MMD has also yielded some provocative results. Migration of these cells into the intima of the internal carotid at the site of stenosis in MMD has been suggested by pathological studies and it is hypothesized that these cells may play a role in both proximal arterial narrowing as well as distal collateral development[67]. CD34+ cells, a subpopulation of endothelial progenitor cells, have been reported to be detected at increased levels in the blood of patients with MMD when compared to healthy controls and also when compared to patients with non-MMD intracranial arterial stenosis[50,74]. However, conflicting data has been reported when specifically looking at CD34+ cells in children, in which another group has reported decreased levels of CD34+ cells in MMD patients relative to matched controls[31]. Adding to the complexity of biomarker development, research has also been undertaken assessing not just the quantity of cells present, but also evaluating their function, as measured by assays of tube formation and colony formation[20,31]. It has been proposed that these circulating endothelial cells exhibit reduced function when assessed *in vitro* relative to those from healthy controls.

The use of living cells as biomarkers for MMD is inherently more complicated than measurement of protein levels or genetic analysis. Variability in isolation of specific subpopulations of cells, the dynamic nature of cell marker expression and the lack of standardization in measures of cell function are technical issues that currently limit the utility of this approach. However,

Table 5. Genetic biomarkers of moyamoya

Genetic associations with moyamoya	Specific mutations linked to moyamoya
Chromosome 3, 6, 8, 17[17,18,21,45,57]	ACTA2 R179[11,48]
HLA-1, -2[12,16]	RNF213 (14576 G>A)[22,41,46]
Neurofibromatosis type I[35,63]	
Sickle cell disease[54,61,63]	
Down syndrome (trisomy 21)[21]	

HLA : human leukocyte antigen, ACTA 2 : Smooth muscle aortic alpha-actin, RNF213 : Ring finger protein 213

the concept of cell-based biomarkers holds tremendous appeal and it is expected that future efforts will mitigate many of these problems. Ultimately, circulating cells may provide the best combination of proteomic, genomic and functional markers of disease.

GENES AND EPIGENETIC MARKERS

The ultimate goal of biomarkers is to unequivocally identify the presence or absence of disease with a high degree of sensitivity and specificity. Discovery of a genetic mutation that is reproducibly linked with a distinct disease phenotype is one the most sought-after objectives in biomedical research. The ability to associate specific genes with MMD is complicated by the likely heterogeneity of disorders that share a common phenotype[58]. Initial efforts to discover genetic markers of MMD reflected this complexity, with a wide range of chromosomes, genes and hereditary diseases reported to be putative markers (Table 5).

While these early attempts may have met with mixed success−and may yet yield important discoveries for individual subtypes of MMD syndrome−two major advances in the genetics of MMD recently been reported and validated by several groups. First, the discovery of R179 mutations in *ACTA2* revealed that specific mutations in genes specific to smooth muscle cells of the vasculature can reliably manifest an arteriopathy identifiable as moyamoya[11,48]. However, it rapidly became apparent that this mutation was associated with only a small minority of MMD cases, as evidenced by studies in Asia and Europe[55,60]. The second major genetic biomarker of MMD was a mutation in the gene for RNF213[22,40,46]. While the function of the protein encoded by the gene remains to be confirmed (potentially a regulator of an ATPase in smooth muscle cells), population studies suggest that this is a major contributor to MMD disease in patients of Asian ancestry, present in up to 90% of familial cases in Asia. Moreover, specific mutations in this gene may also help to improve prognosis, with one base-pair mutation predicting a severe, early-onset form of moyamoya[46].

These new biomarkers are changing the practice of physicians who treat patients with MMD, informing clinical decisions and helping to predict familial risk. While guidelines remain in evolution, the impact of this sort of biomarker discovery is clear. Future efforts will need to refine subgroups of MMD by genetic mutational analysis, define function of relevant genes and evaluate potential epigenetic factors that may serve as important modulators of disease phenotype[52,53].

IMPLEMENTATION AND FUTURE DIRECTIONS

Disease-specific biomarkers are only important if they confer benefits to patients in clinical practice. "The key issue at hand is determining the relationship between any given measurable biomarker and relevant clinical endpoints"[66]. Ideally, biomarkers should aid in the diagnosis, prognosis or treatment of disease. Consequently, discussion of the potential use of MMD-specific biomarkers is a critical factor guiding their development and implementation.

Accurate and timely diagnosis of MMD is critically important. The single factor that overwhelmingly influences long-term outcome is the neurological status at time of treatment[59]. Data from patients with early diagnosis of MMD prior to devastating stroke supports the premise that the ability to make an early diagnosis of the arteriopathy would profoundly improve the outcome of patients[35,40]. As described here, there are several biomarker-related approaches that can directly impact this objective.

Refinement of clinical phenotypes that predict at-risk populations is ongoing and the first step in selecting individuals as candidates for further testing. Imaging remains the gold standard for confirming the diagnosis of MMD, but widespread utilization of imaging studies is cost-prohibitive. In contrast, the development of cheaper, non-invasive screening tests predicated on biomarkers able to detect MMD would revolutionize the care of affected patients. Measurement of proteins in serum, blood or urine would be particularly useful for this goal, as would genetic testing. ELISA and gene sequencing technologies are readily available, relatively low-cost and could markedly complement the use of imaging studies.

The prognosis of MMD continues to challenge physicians. Some populations are prone to rapid, fulminant decline (such as very young infants), while other groups may manifest a far more indolent course[19,36,37,72]. Development of biomarkers to better predict those patients that would benefit from surgery is an area of interest. Radiographic biomarkers may be particularly useful for this objective. For example, data enabling surgeons to prospectively identify higher risk of contralateral progression (in unilateral MMD) or increased likelihood of posterior circulation stroke is already influencing practice patterns[39,40,62].

Biomarkers may also impact care by improving therapeutic efficacy. Studies of cell function may help to predict the capacity for therapeutic angiogenesis, and these data could be used to inform the decision about choosing direct or indirect bypass. Measures of circulating peptides may provide additional data to more accurately predict response to surgery. Ultimately, changes in measured biomarker levels may suggest novel therapeutic agents, such as growth factors that could be used to modulate surgical collateral growth[23,38].

Moving forward, biomarker development for MMD has tremendous potential, but also faces challenges. The relative rarity

of the disease means that collaboration between centers will be important in validating candidate biomarkers. Shared data, longitudinal studies and comparative trials will be crucial to generating meaningful results. Equally important will be efforts to generate consensus on how to best utilize the new data provided by biomarkers. Clear articulation of the strengths and weaknesses of new diagnostic and prognostic capabilities afforded by research will help to avoid unrealistic expectations. Laboratory efforts should be complemented by regular meetings of working groups focused on objectively reviewing progress on biomarker applications in the clinic.

CONCLUSION

MMD-specific biomarkers include clinical phenotypes, radiographic signatures, patterns of protein expression, distinct circulating cell populations and specific genetic mutations. Generally, evidence of characteristic narrowing of the anterior cerebral circulation, reduction in cortical perfusion, elevations in angiogenesis-related peptides and alterations in circulating endothelial cell function are common findings. Several genetic associations have been described, with two recently reported specific mutations (in ACTA2 and RNF213) that manifest distinct clinical presentations. It is increasingly apparent that the term "moyamoya" encompasses many different pathophysiologic conditions and that the use of biomarkers will refine and improve our understanding of this arteriopathy. Future efforts will benefit from multicenter studies and working groups to help guide adoption of utilization in clinical practice.

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