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EDITORIAL COMMENT

Large Transthyretin Aggregates in Plasma of ATTR Amyloidosis Patients

Future Clinical Implications

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Amyloidogenic transthyretin (ATTR) amyloidosis is a relentlessly progressive disease caused by the misfolding and systemic accumulation of amyloidogenic transthyretin into amyloid fibrils that deposit into the different organ and tissues, causing disruption in the tissue architecture and ultimately organ dysfunction. Heart failure and polyneuropathy are the main disease manifestations, with cardiac involvement being the main driver of prognosis. While the structure of the amyloidogenic precursor and the amyloid fibrils is often well characterized, much is still unknown about the transient partially unfolded species involved in the aggregation process, which ultimately lead to the formation of amyloid fibrils. Specifically, the mechanism underlying the conversion of globular proteins from their functional native state to a fibrillar pathological state is yet to be fully understood.

In this issue of *JACC: Basic to Translational Science*, Pedretti et al[1] provide interesting insights into the identification of such amyloidogenic intermediates in the plasma of patients with cardiac ATTR amyloidosis. Using the cryogenic electron microscopy structures of mature ATTR fibrils, the authors developed a peptide probe, named transthyretin aggregation detector 1 (TAD1) for fibril detection. The peptide probe specifically binds large ATTR species. Pedretti et al[1] show that TAD1 targets aggregation-driving segments of transthyretin at the tips of fibrils as well as along the fibril surface in a conformation-dependent manner. They also confirm that TAD1 detects not only ATTR fibrils after purification or in tissue homogenates, but also unique ATTR species in plasma of patients with ATTR amyloidosis. They demonstrate that these species are high-molecular-weight oligomers that are distinct from ATTR oligomers found in neuropathic ATTR amyloidosis patients. Pedretti et al conclude that observations from this study open many questions about the biology of ATTR amyloidosis and reveal a potential diagnostic as well as a therapeutic target. The group are world leaders in the field and should be congratulated for this important work.

The advent of cryogenic electron microscopy (cryo-EM) has exponentially enhanced our understanding of amyloid fibril structures. Until recently, x-ray crystallography was the most common technique used to create 3-dimensional images of biological structures. X-ray crystallography involves arranging the sample into a crystal and shining x-rays through it. The resulting pattern can be used to determine the structure of the individual molecules in the sample. While x-ray crystallography is a good way to determine the structures of specific molecules, it does not capture important information about how these molecules actually behave in and around cells. Cryo-EM allows to capture images of biological molecules without the need to artificially crystallize them. Cryo-EM has revolutionized the structural study of amyloid proteins owing to its exceptional ability to resolve high-resolution structures of individual fibril polymorphs, even within mixtures of diverse fibrillar species and in complex solution environments. The multitude of cryo-EM structures of amyloid fibrils derived from different precursor proteins, deposited in recent years, demonstrate that these fibrils are indeed distinct; therefore, as an example, fibrils

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generated by transthyretin (TTR) differ from those generated by κ or λ light chains. This new evidence has shifted the old paradigm that morphologically indistinguishable amyloid fibrils can be generated from a variety of protein precursors and has opened up the possibility of developing probes and antibodies targeting epitopes exposed in one specific type of amyloid fibril only. ATTR fibrils have revealed a common core with local conformational changes. All cryo-EM structures of ATTR amyloid fibrils consistently exhibit β-strands F and H on the exterior of the fibril surface exposed to the solvent. These strands are critical for transthyretin aggregation in vitro. The structural conformation adopted by these strands in the fibrils significantly differs from that observed in the native TTR precursor. This observation prompted Pedretti et al to develop short peptide probes (TADs), specifically recognizing strands F and H in their pathological conformation.

When probing the plasma of patients with cardiac and mixed phenotype ATTR amyloidosis with one of these TADs, TAD1, Pedretti et al detected large-molecular-weight oligomers, suggesting a complex pathway for TTR amyloidogenesis involving large intermediate aggregates, the origin of which remains undetermined. It is challenging to believe that amyloid fibrils, resistant to treatment with strong acids, bases, and denaturing agents, could spontaneously "shred" and generate large aggregates released into circulation. Thus, de novo nucleation appears to be a more plausible cause for the formation of these circulating high-molecular-weight species.

In vitro evidence supports the hypothesis that factors such as mechanical stress, proteolysis,[2] metal ion concentration,[3] and other mechanisms can induce partial unfolding of amyloidogenic precursors and facilitate amyloidogenesis. However, it remains unknown whether these unfolding conditions occur near the site of amyloid deposition or elsewhere in vivo. The presence of circulating oligomeric species suggests that aggregation-prone intermediates could be generated far from the deposition site and circulate in the bloodstream and deposit when favorable conditions for amyloid deposition are met in specific organs or tissues. Further studies are needed to elucidate the detailed mechanisms and steps involved in the formation of large TTR aggregates.

The detection of large TTR aggregates in plasma has potential profound implications for both diagnosis and treatment. Currently, the diagnosis of ATTR often occurs at a late stage in the disease natural history by which time significant organ damage has already occurred. Typically, patients are diagnosed in the presence of significant heart failure symptoms or peripheral neuropathy and autonomic dysfunction. The ability to detect these large aggregates in plasma could enable the identification of patients at risk of developing ATTR amyloidosis as well as earlier diagnosis in patients with evidence of early amyloid deposition, potentially allowing for timely therapeutic intervention. This is particularly important considering the growing body of evidence that suggests that early initiation of disease-modifying treatment is associated with significantly better prognosis in both patients with ATTR cardiomyopathy and patients with ATTR polyneuropathy.

Furthermore, understanding the structural characteristics of these aggregates could provide not only potential targets for novel therapeutic strategies aimed at preventing TTR misfolding and aggregation, but also strategies that target the removal of amyloid deposits. Halting disease progression at an earlier stage by targeting misfolded, aggregation-prone species could offer new treatment options for patients with ATTR amyloidosis. This could translate to reduction of the rate of disease progression by significantly reducing new amyloid deposition. However, the greatest promise is targeting the removal of existing deposits. There is growing interest in the development of antibodies targeting deposited amyloid, with several ongoing clinical trials in different stages of development, ranging from small proof-of-concept phase 1 studies to large phase 3 placebo control trails. A recent report on 3 patients[4] who, by spontaneously developing immunoglobulin G antibodies to human ATTR amyloid, were able to clear the amyloid from the heart with remodeling to near-normal cardiac structure and function established the unanticipated potential for reversibility of ATTR-CM and raised expectations for its treatment. The study of these plasma ATTR aggregates and their implications in terms of immune response has the potential to inform on immunotherapy strategies in ATTR amyloidosis.

A number of important questions remain: how long before clinically significant amyloid deposition do these large oligomers appear in the bloodstream? Are such aggregates detectable in individuals who are particularly at risk of developing cardiac ATTR amyloidosis, such as those with lumbar canal stenosis or carpal tunnel syndrome, and if so, how long before manifestations of cardiac amyloid become apparent? Are such aggregates detectable in elderly individuals without overt clinical amyloidosis, given that many of them may have clinically silent ATTR amyloid

deposits? Further research is required to investigate the correlation between the presence of these large oligomers and clinical phenotypes, thereby enhancing our understanding of disease susceptibility and behavior. However, these results are indeed promising and could pave the way for identification of novel clinical biomarkers.

In summary, Pedtretti et al[1] have used the structures of ATTR fibrils to design a novel peptide that binds ex vivo ATTR fibrils, transthyretin aggregates generated in vitro, and ATTR species in plasma of ATTR amyloidosis patients. Their findings open many important questions about the biology and pathogenesis of ATTR amyloidosis and may lead to the development of diagnostic assays as well as novel therapeutic targets.

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