Title:

Can staining prostatic samples with Congo Red identify patients at risk for developing TTR Cardiac Amyloidosis.

Study Description:

1. Study purpose and rationale:

Background:

Cardiac amyloidosis is an infiltrative cardiomyopathy caused by infiltration of cardiac myocytes by low molecular weight proteins composed of either immunoglobulin light chains (AL amyloid) or by mis-folded transthyretin (TTR) protein. AL amyloid cardiomyopathy is an exceedingly rare condition caused by clonal plasma cell proliferation and typically leads to an acute onset of heart failure with extremely high mortality with a short survival time.[1] TTR cardiac amyloidosis is an under diagnosed, chronically progressive condition that leads to gradual decline in cardiac function. TTR is a protein composed of four subunits synthesized in the liver that form a tetramer capable of binding thyroid hormone and the retinol-binding protein (making it capable of transporting Vitamin A)[2]. In TTR cardiac amyloidosis there is dissociation of the tetramer with subunit mis-folding and conglomeration into fibrils which can then deposit into various tissues throughout the body[3]. TTR cardiac amyloidosis can result from either wild type-TTR (wtTTR) called senile systematic amyloidosis affecting up to 25% of people 80 years of age or older, or from a genetic variant of the TTR protein with isoleucine substituted for valine at TTR position 122 (v122i allele).[4, 5] Currently, the diagnosis of cardiac amyloidosis begins with clinical suspicion followed by cardiac magnetic resonance imaging and endomyocardial biopsy from a venous approach (citation).[1] No treatment currently exists that significantly slows the progression of TTR cardiac amyloidosis.

The incidence of cardiac amyloidosis leading to symptoms of congestive heart failure is higher in African Americans than other races[6]. This is largely due to the increased incidence of the v122i allele in this population[7]. Cardiac involvement by mis-folded TTR in patients with the v122i allele does not occur until after the age of 60 and does not contribute to cardiac function decline or increased mortality before the age of 65.[8, 9] Recent evidence suggests that small molecules designed to fit into the TTR tetramer may stabilize the tetramer configuration preventing mis-folding of TTR thereby preventing fibril formation (foldrx.com).[3] If these small molecules can prevent fibril formation then theoretically if they are started early enough they could prevent cardiac amyloidosis and the progression to heart failure. These new findings suggest that a reliable screening technique would be useful for early identifying patients with mis-folded TTR prior to development of heart failure symptoms.

African American males are also at increased risk of developing prostate cancer, especially around the age when mis-folded TTR would begin to accumulate in the myocardium. However, prostate biopsies are not routinely evaluated for the presence of amyloid as this requires specialized staining.[10] Recently, we were able to identify amyloid deposits in a prostate biopsy specimen from a 75 year old AA male with urinary obstruction and a phenotype consistent with cardiac amyloidosis.

Hypothesis:

African American men will have a higher incidence of amyloid found on prostatic tissue staining than white males. The majority of amyloid found in African American males will be secondary to TTR and these patients will test positive for the v122i allele.

2. Study design and statistical procedures:

Study Design:

This will be a single center, retrospective, case control study evaluating prostatic tissue from 120 consecutive African American males and 120 consecutive white males 65 years of age or older who underwent either trans-urethral resection of the prostate (TURP), radical prostatectomy (RP), or trans-rectal biopsy for evaluation of prostate cancer or benign prostatic hyperplasia. Prostatic tissue samples will be obtained from the prostatic tissue bank and will be provided for analysis to the study pathologist in a de-identified manner. Prostate biopsy specimens will then be stained for Amyloid using Congo Red or Sulfated Alcian blue. Biopsy samples staining positive for Amyloid deposition will then be analyzed by PCR amplification of the TTR gene to identify the presence of the V122I allele.[11]

Statistical procedures:

Based on an expected amyloid presence of 2% in the white population (secondary to senile amyloidosis) and an expected presence of amyloid of 12% in the African American population (accounting for both senile amyloidosis and v122i TTR amyloidosis) we determined that 120 patients in each study arm would be necessary to achieve an 80% power to detect a 10% difference in presence of amyloid. Furthermore, with 120 African American patients we would have 80% power to detect a 60% difference between Amyloid detected in prostatic tissue and the presence of the v122i allele found on PCR analysis.

We plan to use chi-squared analysis to determine if there is a statistically significant difference in the percentage of amyloid found in prostatic tissue sample from white males and those from African American males.

We then plan to use chi-squared analysis to determine if there is a statistically significant difference between the presence of amyloid found on Congo Red staining and the identification of the v122i allele.

3. Study Procedures:

Parrafin prostatic tissue will be obtained for 120 consecutive African American males and 120 consecutive white males aged 65 years or older as identified in the prostate cancer database maintained by the Department of Urology. Prostate tissue will be located using pathology accession number. Prostatic tissue samples will be evaluated by a single member of the Department of Pathology at NY Presbyerian / Columbia. Each tissue sample from both groups will be stained using Congo Red to evaluate for the presence of amyloid. Samples staining positive for Congo Red will be evaluated for the

presence of TTR protein using immuno-histochemistry. All tissue samples from African American patients will then be tested for the presence of the v122i allele using Sequenom® assay.

4. Study Drugs or Devices:

Not applicable

5. Study Instruments:

Not applicable

6. Study Subjects:

Inclusion Criteria:

- Racial background: 120 consecutive white patients, 120 consecutive African American patients
- Underwent either trans-urethral resection of the prostate, radical prostatectomy, or prostate biopsy for evaluation of elevated PSA, abnormal physical exam finding, follow up for known prostate cancer, therapy for benign prostatic hyperplasia, or other prostatic pathology
- Procedure was performed at NY Presbyterian / Columbia

Exclusion Criteria:

- Age: any patient < 65 years of age
- Inadequate tissue sample size for pathologic evaluation

7. Recruitment:

Eligible patients will be identified from a secure, HIPPA compliant database maintained by the Department of Urology. There will be no patient recruitment.

8. Informed Consent Process:

Please refer to Waiver of Authorization

9. Confidentiality of Study Data:

Eligible patients will be identified from a data repository maintained by the Department of Urology, stored in a secure, HIPPA compliant, password protected database on a secure server within the department of urology. Pathology accession numbers will used to obtain paraffin prostatic tissue samples. Prostatic tissue will be delivered to the study pathologist identified only by pathology accession number. A unique study identification number will be assigned to each sample. No unique identifiers will be available to study participants at any time. At no point will unique identifiers be printed into a physical paper copy or saved to a local computer.

10. Privacy Protection:

Protected health information (PHI) will not be used for the purposes of this study. PHI will not be disclosed to any of the study participants and will not be disclosed to any other person or entity, except as required by law for authorized oversight of the research study. Prostatic tissue staining positive for amyloid will not be able to be linked to any PHI by any study investigators. Study investigators will be unable to contact patients or any patient's family members or primary care physicians.

11. Potential Risks:

There are no potential direct risks to patients involved in this study. This is a retrospective study that will use a coded data set.

12. Data and safety monitoring:

Since PHI will not be available for prostatic tissue samples, we anticipate minimal to no risk to study participants.

13. Potential benefits:

There are unlikely to be any direct benefits to patients selected to be in this study. We hope that this research will provide information that may eventually lead to a reliable means of establishing an early diagnosis for TTR amyloidosis.

14. Alternatives:

Not applicable

15. Research at External Sites:

Not applicable

16. Columbia at lead institution:

Not applicable

References

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Appendix 1: Lay Abstract:

In the condition called “cardiac amyloidosis” small proteins in the blood stream can get stuck inside the heart leading to difficulty breathing, difficulty walking, and can eventually lead to death from a condition called “heart failure”. These proteins can also get stuck in other parts of the body, for example the prostate. One type of this condition is passed down from family members in genes and occurs almost only in African Americans. African Americans are also at increased risk of developing problems with their prostate around the same age that these proteins are causing damage to the heart. We intend to review prostate samples to look for the presence of the proteins that can cause damage to the heart. We hypothesize that we will be able to find the same proteins that cause damage to the heart by looking at the prostate tissue. This will allow us to identify people that are at risk for developing “heart failure”.

Appendix 2: Scientific Abstract:

TTR (transthyretin) cardiac amyloidosis (ATTR) is a disease primarily of older adult males. Diagnosis requires pathological confirmation of amyloid. In the United States, ~4% of African Americans (AA) have a mutation in the transthyretin gene (V122I, substitution of valine for isolucine) which is autosomal dominant and causes ATTR cardiac amyloidosis. Since ATTR and prostatic disease are over-represented in AA males, we hypothesize the diagnosis could be made by examining prostatic tissue.