Kidney Medicine

Donor-Derived ALECT2 Amyloidosis and Recurrent Fibrillary Glomerulonephritis in a Transplant Allograft

Samih H. Nasr, Octavio Chavez, Surendra Dasari, Jason D. Theis, Julie A. Vrana, Huma Fatima, Liying Fu, Rajendra S. Baliga, and Ellen D. McPhail

The occurrence of renal amyloidosis and fibrillary glomerulonephritis in the same biopsy specimen is exceptional and poses a diagnostic challenge. We describe the case of a non-Hispanic White patient with end-stage kidney disease due to fibrillary glomerulonephritis who received a second living donor kidney from a Hispanic individual. A 40-month–posttransplantation biopsy performed for an elevated serum creatinine level revealed interstitial congophilic deposits and glomerular noncongophilic fibrillary deposits, in addition to rejection. Separate laser microdissections of the glomerular and interstitial deposits followed by liquid chromatography–tandem mass spectrometry (LC MS/MS) revealed DNAJB9 peptide spectra in glomeruli and a peptide profile consistent with leukocyte chemotactic factor 2 (ALECT2) amyloidosis in the interstitium. Based on these findings, a 2-week–posttransplantation biopsy was re-reviewed and analyzed using LC MS/MS, which revealed a peptide profile consistent with ALECT2 amyloidosis in the interstitium, without peptide spectra for ALECT2 or DNAJB9 in glomeruli. The findings were consistent with donor-derived ALECT2 amyloidosis and recurrent fibrillary glomerulonephritis. At 49 months post-transplantation, allograft function was stable with minimal proteinuria. Thus, LC MS/MS was crucial to establish the accurate diagnosis of these 2 nephropathies characterized by fibrillary deposits. The indolent posttransplantation course suggests that donated kidneys with focal interstitial ALECT2 deposits may be suitable for transplantation but the deposits persist for many years.

Complete author and article information provided before references.

Kidney Med. 3(3):433-437. Published online February 16, 2021.

doi: 10.1016/j.xkme.2020.11.019

INTRODUCTION

Leukocyte chemotactic factor 2 (ALECT2) amyloidosis is a recently described form of amyloidosis[1] that typically manifests as chronic decreased kidney function with bland urinary sediment,[2,3] although postmortem analysis revealed consistent involvement of liver, spleen, lungs, and adrenal glands,[4] usually subclinical. Contrary to other types of amyloidosis, it has a strong bias toward certain ethnic groups, including Hispanics of Mexican origin, Egyptians, and Punjabs, whereas it is very rare in non-Hispanic White populations.[2,3,5-7] Pathogenesis is still unknown, but it could be due to leukocyte cell–derived chemotaxin 2 overexpression by hepatocytes.[5,7] Overall survival is excellent due to the absence of heart involvement, whereas kidney survival is guarded although better than that of other types of amyloidosis.[2,3,5,8] There is no known therapy, aside from kidney transplantation. ALECT2 amyloidosis has been only rarely described in the kidney allograft.[3,9,10]

Fibrillary glomerulonephritis (FGN) was historically defined by glomerular deposition of Congo red–negative, randomly oriented fibrils that stain with antisera to immunoglobulin G (IgG),[11,12] although recent series documented that some cases are Congo red-positive[13,14] and/or IgG-negative.[15] The fibrils of FGN resemble amyloid fibrils but are about twice as large, although there is a significant overlap and thus fibril diameter should not be solely relied on to make this distinction. The recent identification by laser microdissection–assisted liquid chromatography–tandem mass spectrometry (LC MS/MS) of DNAJB9 as a sensitive and specific marker for FGN has revolutionized the diagnosis of this disease[16-18] and now allows for distinction from amyloidosis (including the distinction between congophilic FGN and amyloidosis)[13,14] and other glomerulopathies characterized by organized deposits.[16,17] As in ALECT2 amyloidosis, there is no effective treatment for FGN aside from kidney transplantation, which is associated with disease recurrence in some patients.[14]

Although the occurrence of 2 types of amyloidosis in the same patient has been reported,[19] the presence of amyloidosis and FGN in the same kidney biopsy specimen has not been previously described to our knowledge. We describe an unusual case of donor-derived ALECT2 amyloidosis concurrent with recurrent FGN in the kidney allograft. Laser microdissection of separate anatomic sites of the kidney followed by LC MS/MS was crucial to establish the accurate diagnosis of these 2 nephropathies characterized by randomly oriented fibrillary deposits.

CASE REPORT

The patient was a non-Hispanic man in his early 50s with end-stage kidney disease secondary to Congo red–negative FGN (Fig 1A) who received a living-unrelated donor kidney transplant from a Hispanic man in his early 50s. The patient had a previous kidney transplant from his brother 7 years prior that was lost due to recurrent disease 6 years postimplantation. Pretransplantation evaluation of the second transplant revealed panel-reactive antibody values for HLA antigen class I of 8% and HLA antigen class II of 8%, a negative cross-match, and 3AB2DR HLA antigen

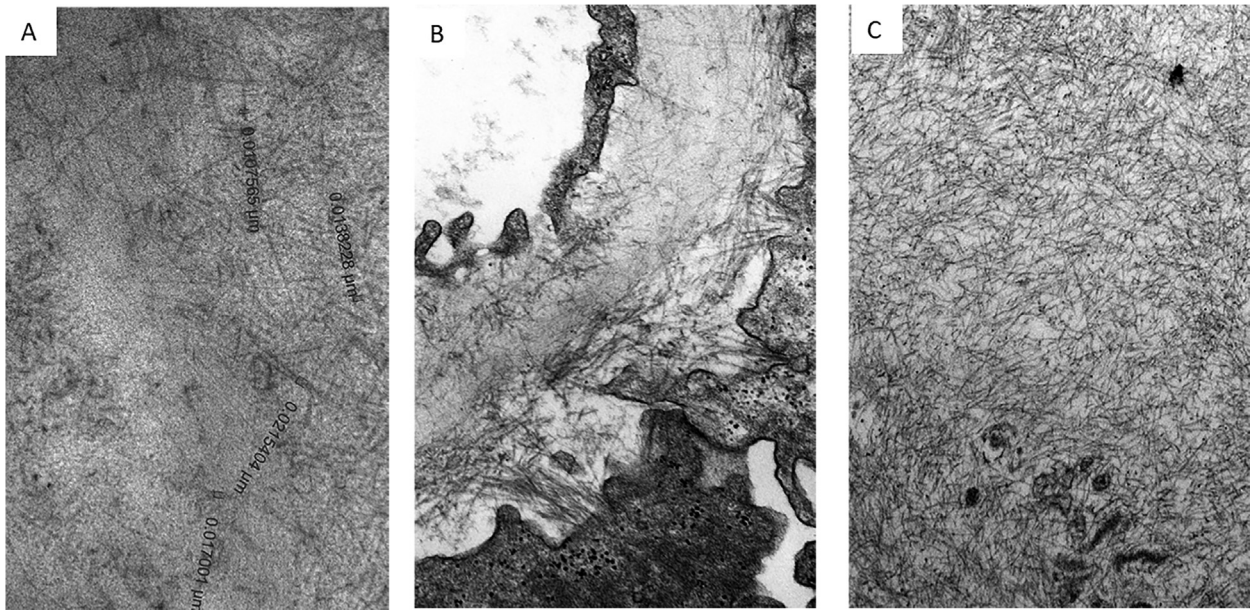

Figure 1. Electron microscopy findings in the native kidney and transplant kidney. (A) An electron microscopy image from the native biopsy shows randomly oriented straight fibrils that permeate the mesangium. The fibrils had a mean diameter of 15 (range, 9-21) nm (original magnification; ×40,000). Similar fibrils were also seen segmentally infiltrating the glomerular basement membranes (not shown). (B) An electron microscopy image from the biopsy of the second allograft, 3.3 years postimplantation, shows randomly oriented straight fibrils that permeate the glomerular basement membrane. The fibrils had a mean diameter of 15 (range 8-22) nm (original magnification, ×50,000). Similar fibrils were also seen segmentally infiltrating the mesangium (not shown). (C) An electron microscopy image from the same allograft biopsy as B shows randomly oriented straight fibrils that permeate the cortical interstitium. Compared with glomerular fibrillary glomerulonephritis fibril, these amyloid fibrils were smaller (mean diameter, 10, range, 7-15 nm) and more closely packed (original magnification, ×50,000).

mismatches with the donor. His induction therapy consisted of thymoglobulin and methylprednisolone and his maintenance therapy consisted of tacrolimus and mycophenolate mofetil (steroid-free regimen).

Two weeks postimplantation, the patient's serum creatinine level acutely increased from 2.3 on discharge to 5.5 mg/dL, which prompted a kidney biopsy that showed acute cellular rejection Banff grade 2A (without evidence of recurrent disease by electron microscopy), which was successfully treated with methylprednisolone and thymoglobulin. Random urinary protein-creatinine ratio was 540 mg/g.

Forty months posttransplantation, the patient was noted to have a serum creatinine level of 1.5 mg/dL, which was elevated above baseline. Urinalysis showed trace blood, and random urinary protein-creatinine ratio was 918 mg/g. There was no evidence of obstruction, tacrolimus level was within the therapeutic range, BK virus DNA was not detected in plasma using reverse transcriptase–polymerase chain reaction, and donor-specific antibody panel was negative. A kidney biopsy was performed.

The biopsy showed features of active antibody-mediated rejection with peritubular capillaritis (ptc3), mild glomerulitis (g1), and diffuse C4d positivity in peritubular capillaries (C4d3) and features suspicious for concurrent acute cellular rejection (Banff grade borderline: i1,t1,v0). There was mild mesangial expansion and hypercellularity (Fig 2A). Immunofluorescence revealed glomerular deposition of IgG (2+), IgM (trace), C3 (1+), C1q (1+), κ (1+), and λ (2+). On electron microscopy, randomly oriented straight fibrils measuring 15 nm in mean thickness were seen permeating the mesangial matrix and segmentally the glomerular basement membrane (Fig 1B), similar to the native kidney biopsy (Fig 1A). In addition, smaller (10 nm in mean thickness) randomly oriented straight fibrils were seen permeating the cortical interstitium (Fig 1C). Congo red stain was negative in glomeruli but was positive in the interstitium, with anomalous colors under polarized light (Fig 2B and C).

To further characterize the glomerular and interstitial deposits, we performed separate laser microdissections of the Congo red–negative glomerular deposits and the Congo red–positive interstitial deposits, followed by LC MS/MS. In glomeruli, abundant peptide spectra corresponding to DNAJB9 were detected, consistent with recurrent FGN, without the peptide profile of amyloidosis (Fig 3). In contrast, in the congophilic interstitial deposits, a peptide profile consistent with ALECT2 was detected without spectra for DNAJB9 (Fig 3). Based on these findings, the paraffin block of the 2-week posttransplantation biopsy was obtained, and sections were stained with Congo red and analyzed using LC MS/MS. Patchy Congo red–positive amyloid deposits were seen in the interstitium, which by LC MS/MS exhibited a peptide profile

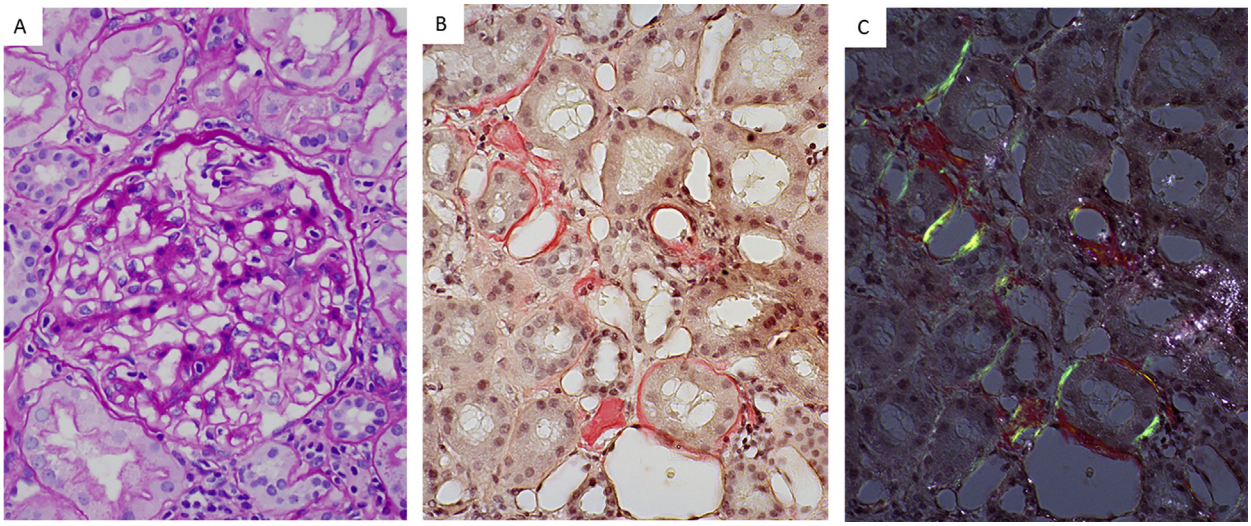

Figure 2. Light microscopy findings in the biopsy of the second allograft. (A) The glomerulus shown exhibits mild global mesangial hypercellularity and matrix expansion (periodic acid–Schiff stain). (B) Interstitial and tubular basement membrane congophilic amyloid deposits. (C) The congophilic deposits show anomalous colors (so called "apple green birefringence") under polarized light (A-C: original magnification, ×400).

Probability Legend:
over 95%
80% to 94%
50% to 79%
20% to 49%
0% to 19%

Bio View:
Identified Proteins (157/161)
Including 1 Decoy

#	Visible?	Starred?		Accession Number	Molecular Weight	Protein Grouping Ambiguity	Interstitial-Sample#1	Interstitial-Sample#2	Glomeruli-Sample#1
1	☑	☑	Leukocyte cell-derived chemotax...	LECT2_HUM...	16 kDa		43	44	
2	☑	☑	DnaJ homolog subfamily B memb...	DNJB9_HU...	26 kDa		3	3	30
3	☑	☑	Apolipoprotein E	APOE_HUM...	36 kDa		32	32	12
4	☑	☑	Serum amyloid P-component	SAMP_HUM...	25 kDa		6	6	1
5	☑	☑	Actin, cytoplasmic 1	ACTB_HUM...	42 kDa	★	95	91	183
6	☑	☑	Vimentin	VIME_HUM...	54 kDa	★	33	33	138
7	☑	☑	Hydroxymethylglutaryl-CoA synt...	HMCS2_HU...	57 kDa		76	74	
8	☑	☑	Vitronectin	VTNC_HUM...	54 kDa		52	53	12
9	☑	☑	3-ketoacyl-CoA thiolase, mitocho...	THIM_HUM...	42 kDa		57	63	
10	☑	☑	Collagen alpha-3(VI) chain	CO6A3_HU...	344 kDa		41	30	34

Figure 3. Proteomics identifies ALECT2 (leukocyte chemotactic factor 2)-type amyloidosis and fibrillary glomerulonephritis biomarkers in different anatomic compartments in the patient's renal biopsy: separate laser microdissections followed by liquid chromatography–tandem mass spectrometry (LC MS/MS) were performed on Congo red–positive interstitial deposits and Congo red–negative glomeruli. The protein identification profile from all samples is shown. Numbers in green boxes show the total number of MS/MS spectral counts associated with each protein in the corresponding sample. MS/MS spectral counts are a surrogate measure of protein abundance in the sample.[21] Proteins with MS/MS counts of 5 or higher are considered for clinical interpretation, and at least 2 universal amyloid tissue biomarkers must be present to verify a diagnosis of amyloidosis. The interstitial deposits (columns 1 and 2) contain the universal amyloid tissue biomarkers apolipoprotein E (APOE) and serum amyloid P-component (SAMP), as well as LECT2 protein, which is the type-defining marker for ALECT2-type amyloid. The glomerular deposits (column 3) lack the proteomic features of amyloidosis but instead contain abundant spectral counts for DNAJB9, which is a biomarker for fibrillary glomerulonephritis.

consistent with ALECT2. No spectra for DNAJB9 or a peptide profile of ALECT2 were detected in glomeruli.

The patient was treated with methylprednisolone, plasmapheresis, and intravenous immunoglobulin, followed by prednisone taper. Nine months later (49 months posttransplantation), serum creatinine level was 1.56 mg/dL and random urinary protein-creatinine ratio was 658 mg/g. The donor's most recent serum creatinine level and random urinary protein-creatinine ratio, 51 months postdonation, are 1.31 mg/dL and 141 mg/g, respectively.

DISCUSSION

The incidence of ALECT2 amyloidosis in kidney allograft biopsies has not been investigated. Anecdotal reports showed that it can be of donor origin[3,9] or de novo.[10] Recurrence of renal ALECT2 amyloidosis is questionable. In our previous report of 72 patients with ALECT2 amyloidosis, 5 received a kidney transplant.[3] In one of the patients who received a living related donor kidney from his niece, amyloid deposits were detected on a 6-month posttransplantation biopsy, which we interpreted as evidence of disease recurrence.[3] However, several years later, the donor's father (ie, recipient brother) had renal ALECT2 amyloidosis diagnosed. Thus, retrospectively, that case likely represents familial donor-derived (rather than recurrent) ALECT2 amyloidosis (S.H.N., unpublished data).

In the current case, a non-Hispanic White patient received a kidney from a Hispanic individual and a 2-week posttransplantation biopsy revealed interstitial ALECT2 amyloidosis; thus, ALECT2 is likely donor derived. The recipient's ethnicity and short time from implantation to amyloid detection argue against de novo disease and the absence of ALECT2 in the native biopsy and first allograft argue against recurrent disease. However, the patient had recurrent FGN involving glomeruli but not the interstitium. In a series from our center of 14 patients with FGN who underwent kidney transplantation and protocol allograft biopsies (which included the current case), only 3 (21%) had histologic evidence of recurrence, detected 5 to 10 years posttransplantation, and was associated with an indolent course.[20] Interestingly, our patient who lost his first allograft due to recurrent FGN had stable second-allograft function more than 4 years postimplantation with only minimal proteinuria, despite having FGN, ALECT2 amyloidosis, and rejection. Thus, both donor-derived ALECT2 amyloidosis and recurrent FGN in the second allograft could be subclinical.

The exceptional development of amyloidosis and FGN in the same biopsy specimen poses a diagnostic challenge to the pathologist due to the ultrastructural similarities of these diseases and the fact that deposits in some FGN cases are Congo red-positive[13,14] and/or IgG-negative.[15] Separate laser microdissections of glomerular and interstitial deposits followed by LC MS/MS were crucial to establish the accurate diagnosis of these 2 nephropathies. However, this technique is not widely available. Alternatively, the combination of DNAJB9 and LECT2 immunohistochemical stains could be used to establish the diagnosis. The indolent posttransplantation course supports that donated kidneys with focal ALECT2 deposits may be suitable for transplantation but the deposits in the allograft persist for many years. The impact of kidney donation in individuals with subclinical ALECT2 amyloidosis remains to be determined.

ARTICLE INFORMATION

Authors' Full Names and Academic Degrees: Samih H. Nasr, MD, Octavio Chavez, MD, Surendra Dasari, PhD, Jason D. Theis, BS, Julie A. Vrana, PhD, Huma Fatima, MD, Liying Fu, MD, Rajendra S. Baliga, MD, and Ellen D. McPhail, MD.

Authors' Affiliations: Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN (SHN, JDT, JAV, EDM); Tampa General Hospital, Tampa, FL (OC, LF, RSB); Department of Health Sciences Research, Mayo Clinic, Rochester, MN (SD); and Department of Pathology, University of Alabama at Birmingham, Birmingham, AL (HF).

Address for Correspondence: Ellen D. McPhail, MD, Division of Hematopathology, Mayo Clinic, 200 First St SW, Rochester, MN 55905. E-mail: mcphail.ellen@mayo.edu

Support: None.

Financial Disclosure: The authors declare that they have no relevant financial interests.

Patient Protections: The authors declare that they have obtained consent from the patient reported in this article for publication of the information about him that appears within this Case Report and any associated supplementary material.

Peer Review: Received July 28, 2020. Evaluated by 3 external peer reviewers, with direct editorial input from an Associate Editor and the Editor-in-Chief. Accepted in revised form November 21, 2020.

REFERENCES

1. Benson MD, James S, Scott K, Liepnieks JJ, Kluve-Beckerman B. Leukocyte chemotactic factor 2: a novel renal amyloid protein. *Kidney Int*. 2008;74(2):218-222.
2. Larsen CP, Kossmann RJ, Beggs ML, Solomon A, Walker PD. Clinical, morphologic, and genetic features of renal leukocyte chemotactic factor 2 amyloidosis. *Kidney Int*. 2014;86(2):378-382.
3. Said SM, Sethi S, Valeri AM, et al. Characterization and outcomes of renal leukocyte chemotactic factor 2-associated amyloidosis. *Kidney Int*. 2014;86(2):370-377.
4. Larsen CP, Beggs ML, Wilson JD, Lathrop SL. Prevalence and organ distribution of leukocyte chemotactic factor 2 amyloidosis (ALECT2) among decedents in New Mexico. *Amyloid*. 2016;23(2):119-123.
5. Nasr SH, Dogan A, Larsen CP. Leukocyte cell-derived chemotaxin 2-associated amyloidosis: a recently recognized disease with distinct clinicopathologic characteristics. *Clin J Am Soc Nephrol*. 2015;10(11):2084-2093.
6. Larsen CP, Ismail W, Kurtin PJ, Vrana JA, Dasari S, Nasr SH. Leukocyte chemotactic factor 2 amyloidosis (ALECT2) is a common form of renal amyloidosis among Egyptians. *Mod Pathol*. 2016;29(4):416-420.
7. Mereuta OM, Theis JD, Vrana JA, et al. Leukocyte cell-derived chemotaxin 2 (LECT2)-associated amyloidosis is a frequent

cause of hepatic amyloidosis in the United States. *Blood*. 2014;123(10):1479-1482.
8. Rezk T, Gilbertson JA, Rowczenio D, et al. Diagnosis, pathogenesis and outcome in leucocyte chemotactic factor 2 (ALECT2) amyloidosis. *Nephrol Dial Transplant*. 2018;33(2):241-247.
9. Mejia-Vilet JM, Cardenas-Mastrascusa LR, Palacios-Cebreros EJ, et al. LECT2 amyloidosis in kidney transplantation: a report of 5 cases. *Am J Kidney Dis*. 2019;74(4):563-566.
10. Shamir ER, Lee MM, Walavalkar V. De novo leukocyte chemotactic factor 2 amyloidosis in a pediatric renal allograft, 15 years post-transplant. *Pediatr Transplant*. 2019;23(3):e13371.
11. Bridoux F, Hugue V, Coldefy O, et al. Fibrillary glomerulonephritis and immunotactoid (microtubular) glomerulopathy are associated with distinct immunologic features. *Kidney Int*. 2002;62(5):1764-1775.
12. Rosenstock JL, Markowitz GS, Valeri AM, Sacchi G, Appel GB, D'Agati VD. Fibrillary and immunotactoid glomerulonephritis: Distinct entities with different clinical and pathologic features. *Kidney Int*. 2003;63(4):1450-1461.
13. Alexander MP, Dasari S, Vrana JA, et al. Congophilic fibrillary glomerulonephritis: a case series. *Am J Kidney Dis*. 2018;72(3):325-336.
14. Andeen NK, Troxell ML, Riazy M, et al. Fibrillary glomerulonephritis: clinicopathologic features and atypical cases from a multi-institutional cohort. *Clin J Am Soc Nephrol*. 2019;14(12):1741-1750.
15. Said SM, Best Rocha A, Royal V, et al. Immunoglobulin-negative DNAJB9-associated fibrillary glomerulonephritis: a report of 9 cases. *Am J Kidney Dis*. 2021;77(3):454-458.
16. Nasr SH, Vrana JA, Dasari S, et al. DNAJB9 is a specific immunohistochemical marker for fibrillary glomerulonephritis. *Kidney Int Rep*. 2017;3(1):56-64.
17. Andeen NK, Yang HY, Dai DF, MacCoss MJ, Smith KD. DnaJ homolog subfamily B member 9 is a putative autoantigen in fibrillary GN. *J Am Soc Nephrol*. 2018;29(1):231-239.
18. Dasari S, Alexander MP, Vrana JA, et al. DnaJ heat shock protein family B member 9 is a novel biomarker for fibrillary GN. *J Am Soc Nephrol*. 2018;29(1):51-56.
19. Sidiqi MH, McPhail ED, Theis JD, et al. Two types of amyloidosis presenting in a single patient: a case series. *Blood Cancer J*. 2019;9(3):30.
20. El Ters M, Bobart SA, Cornell LD, et al. Recurrence of DNAJB9-positive fibrillary glomerulonephritis after kidney transplantation: a case series. *Am J Kidney Dis*. 2020;76(4):500-510.
21. Liu H, Sadygov RG, Yates JR 3rd. A model for random sampling and estimation of relative protein abundance in shotgun proteomics. *Anal Chem*. 2004;76(14):4193-4201.