C4d-expressing glomerulopathy and proteinuria post transplantation of a too-big-for-size mismatched kidney allograft: An unusual case with good outcome

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Abstract. A 5-year-old severely growth-retarded child with tubulointerstitial, oliguric end-stage renal disease received an adult-size kidney transplant. Three years post grafting under standard triple immunosuppression (mycophenolate mofetil, tacrolimus, and prednisone) de novo nephrotic range proteinuria without the nephrotic syndrome developed. Graft function was normal (serum creatinine: 0.2 – 0.3 mg/dL), there were no donor-specific HLA antibodies (DSA), and the urine sediment was inactive. Two biopsies collected 3 and 4 years post-transplantation showed severe glomerular capillary wall remodeling and associated pseudolinear C4d staining as morphologic correlates for the proteinuria. Changes resembled those seen in so-called "size-mismatch transplant glomerulopathies". There was no evidence of a glomerulonephritis, acute or chronic rejection including transplant glomerulopathy, interstitial fibrosis, peritubular capillary C4d deposits, or multilamination of peritubular capillary basement membranes. The glomerular changes were not detected in the implantation zero-hour biopsy or the recipient's native renal biopsy. At the end of follow-up 64 months post transplantation, proteinuria persisted at subnephrotic levels in the setting of stable graft function and undetectable DSAs. This unique case adds to the list of causes of nonrejection-associated post-transplant proteinuria. It demonstrates for the first time that a too-large-for-body-size mismatched graft is associated with a presumably sheer stress-induced C4d expressing glomerulopathy, severe proteinuria, and favorable outcome.

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Introduction

Proteinuria post renal transplantation constitutes a clinical alarm for adverse intra-graft events. Depending on the timeline and the degree of proteinuria, three major underlying etiologies can be considered: [1] acute/chronic rejection, [2] recurrent or de novo glomerular diseases (e.g., membranous glomerulopathy, FSGS), [3] other glomerulopathies (e.g., calcineurin inhibitor-induced structural toxicity). Outcome depends on the underlying etiology and is often unfavorable, such as in rejection induced transplant glomerulopathy or recurrent FSGS [1, 2, 3]. Glomerular injury and proteinuria due to transplantation of a severely size-mismatched organ is uncommon. There are previous reports of adult renal allograft recipients with too-small-for-body-size pediatric donor organs presenting with severe proteinuria. The here-described opposite clinical scenario, i.e., proteinuria and favorable outcome in a too-big-for-body-size kidney transplant showing a de-novo, complement factor C4d expressing "size mismatch" glomerulopathy, has hitherto not been described. Our case furthers knowledge of potential complications associated with transplantation of size-mismatched renal allografts [4].

Case report

The patient is an 11-year-old Caucasian female with dwarfism due to heterozygous mutation of the *WDR19* gene, which is linked to cranioectodermal dysplasia 4 (OMIM – Online Mendelian Inheritance in Man: 614378), a syndrome associated with small stature and nephronophthisis. At age

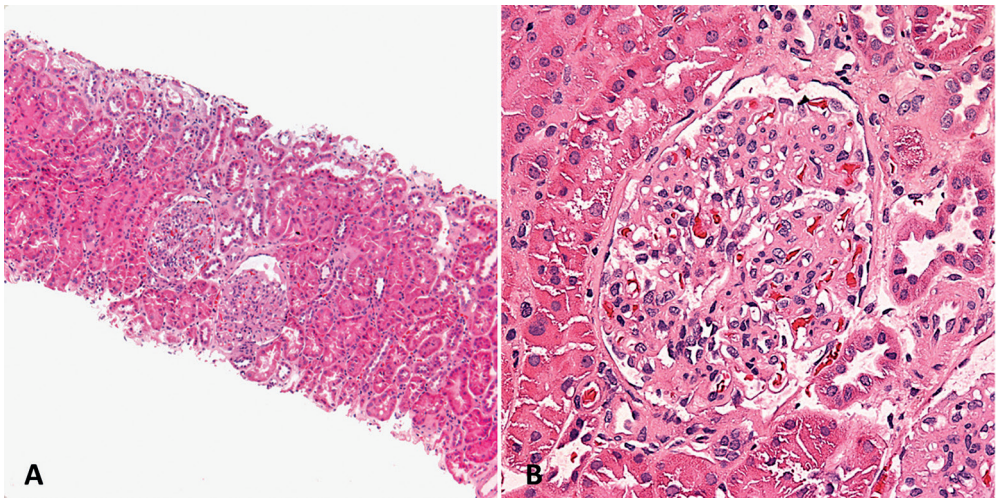

Figure 1. Second follow-up transplant biopsy (2016) showing mild mesangial matrix expansion without interstitial inflammation and only minimal focal fibrosis without tubular atrophy (H & E stain). Identical changes were seen in the first biopsy collected in 2015. Original magnification A: × 200, B: × 600.

3, she presented with progressive renal insufficiency. A native renal biopsy showed advanced chronic changes with 50% global glomerulosclerosis and severe interstitial fibrosis. Immunofluorescence and electron microscopic studies showed no characteristic and diagnostic glomerular abnormalities.

In 2012, at age 5, the by-then oliguric patient underwent renal transplantation with an adult-size organ from a deceased 13-year-old male gunshot victim. The 11.8 kg recipient measured 88.7 cm in height; measurements well below the 3rd percentile for her age. On ultrasound examination, her 4.4 cm and 4.7 cm native kidneys were echo dense with small cysts; (expected kidney length for age: 8.1 cm). The donor organ measured 11.2 cm, which is within the range of a fully matured adult kidney. To accommodate the large donor organ, the allograft was placed intra-abdominally and anastomosed directly to the recipient's aorta. Native nephrectomies were not performed. A post-perfusion zero-hour allograft biopsy showed only mild acute tubular injury without glomerular abnormalities by light, electron, or immunofluorescence microscopy including unremarkable incubations for C4d.

The immediate post-operative course was uneventful with good renal function (serum creatinine (S-Cr): 0.2 – 0.3 mg/dL; transient evidence of hyperfiltration with 165 mL/min estimated glomerular filtration rate (eGFR) calculated by CKiDs bedside Schwartz Formula) under maintenance immunosuppression with mycophenolate mofetil, tacrolimus, and prednisone. During the first year of follow-up (S-Cr: 0.4 mg/dL; eGFR 98 mL/min), the patient showed minimal to mild (1+) proteinuria. Proteinuria increased to moderate levels during year 2 (urine protein to creatinine ratio (UPC): 1.7; S-Cr: 0.3 mg/dL; eGFR 131 mL/min) and reached nephrotic range 3 years post grafting (total urine protein 8.1 g/24 hours; non-nephritic urine sediment; S-Cr: 0.5 mg/dL; eGFR: 88 mL/min). There was no evidence of the nephrotic syndrome, and all standard laboratory test results were within normal limits, including serum albumin and cholesterol levels. Donor-specific MHC-class I or class II antibodies were not detected (also see below). The patient was compliant with her medication with satisfactory tacrolimus trough levels. In 2015 3 years post transplantation, the patient underwent a diagnostic renal allograft biopsy to evaluate the cause of the proteinuria (Figure 1) (Table 1).

Light microscopy showed 16 glomeruli with mild mesangial expansion and a secondary form of segmental tuft sclerosis in 1/16 glomeruli. Capillary loops were within normal limits. The interstitium demonstrated only focal minimal fibrosis and tubular atrophy without inflammation. Blood vessels were unremarkable.

Immunofluorescence microscopy showed, along glomerular capillary walls, diffuse

Table 1. Summary of biopsy and clinical data.

	2012 biopsy (implantation)	2015 biopsy (3 years post grafting)	2016 biopsy (4 years post grafting)
Clinical data			
Proteinuria	N/A	8.1 g/24 hours	4.2 g/24 hours
Hematuria	N/A	Microscopic, no casts	Microscopic, no casts
Serum creatinine	N/A	0.5 mg/dL	0.60 mg/dL
Donor-specific HLA antibodies	N/A	Not detected	Not detected
Nephrotic syndrome	N/A	Absent	Absent
Light microscopy			
Glomeruli	Normal	Minimal non-diagnostic changes	Minimal non-diagnostic changes
Interstitium	Mild acute tubular injury	Minimal interstitial fibrosis, no inflammation	Minimal interstitial fibrosis, no inflammation
Vessels	Normal	Normal, no endarteritis	Normal, no endarteritis
Immunofluorescence			
C4d (glomeruli)	Negative	3+ (global, pseudolinear)	3 – 4+ (global, pseudolinear)
C4d (PTC)	Negative	Negative	Negative
IgM (glomeruli)	Negative	2+ (segmental, granular)	2+ (segmental, granular)
Collagen IV; α5 (glomeruli)	N/A	Normal	Normal
Electron microscopy			
Glomeruli	Normal	Marked transmembranous capillary wall remodeling	Marked transmembranous capillary wall remodeling
Peritubular capillaries	Normal	Normal, no lamination	Normal, no lamination

PTC = peritubular capillaries; N/A = not applicable.

global pseudolinear staining for the complement degradation product C4d (3+ on a scale 0 – 4), diffuse segmentally-accentuated granular staining for IgM (2+ on a scale 0 – 4) and C3 (1+ on a scale 0 – 4) (Figure 2). No characteristic deposits were seen in immunofluorescence incubations to detect IgG, IgA, C1q, fibrin, κ-, and λ-light chains. Staining for collagen type 4, α5 chain demonstrated a normal pattern. C4d staining was not detected along peritubular capillaries. The overall immunofluorescence C4d staining profile (negative along peritubular capillaries, positive along glomerular capillary walls) was confirmed by immunohistochemistry performed on formalin-fixed and paraffin-embedded tissue sections (Figure 3).

Ultrastructurally, diffuse marked irregularities of the glomerular capillary walls were found with splitting/basket weaving of the lamina densa and pronounced remodeling, accentuated under activated podocytes (Figure 4). There was no evidence of capillary wall duplication/transplant glomerulopathy, endocapillary hypercellularity, or immune complex type deposits. Peritubular capillaries did not show basement membrane multilaminations.

A diagnosis of "size-mismatch type glomerulopathy" was rendered. During follow-up, patient management and therapy remained largely unchanged except for the substitution of a calcium channel blocker with a β-blocker for optimized blood pressure control. Renin angiotensin aldosterone system blocking agents were not administered due to a patient history of adverse reactions.

In 2016, 4 years after transplantation, a second follow-up renal biopsy was performed to re-evaluate the cause of persistent nephrotic range proteinuria and a bump in S-Cr levels (4.2 g/24 hours; S-Cr: 0.6 mg/dL; eGFR 75 mL/min). All other clinical and laboratory parameters had remained unchanged. The follow-up biopsy showed light, immunofluorescence, and electron microscopic changes similar to those noted in the biopsy from 2015 (Figures 1, 2, 3, 4) (Table 1); there was no rejection and no increase in chronic injury. Post-biopsy therapy remained unchanged.

At last follow-up, 64 months post grafting, the 11-year-old still severely growth-retarded patient presented with persistent although decreased proteinuria (UP/C of 1.3, urine albumin to creatinine ratio (UA/C) of

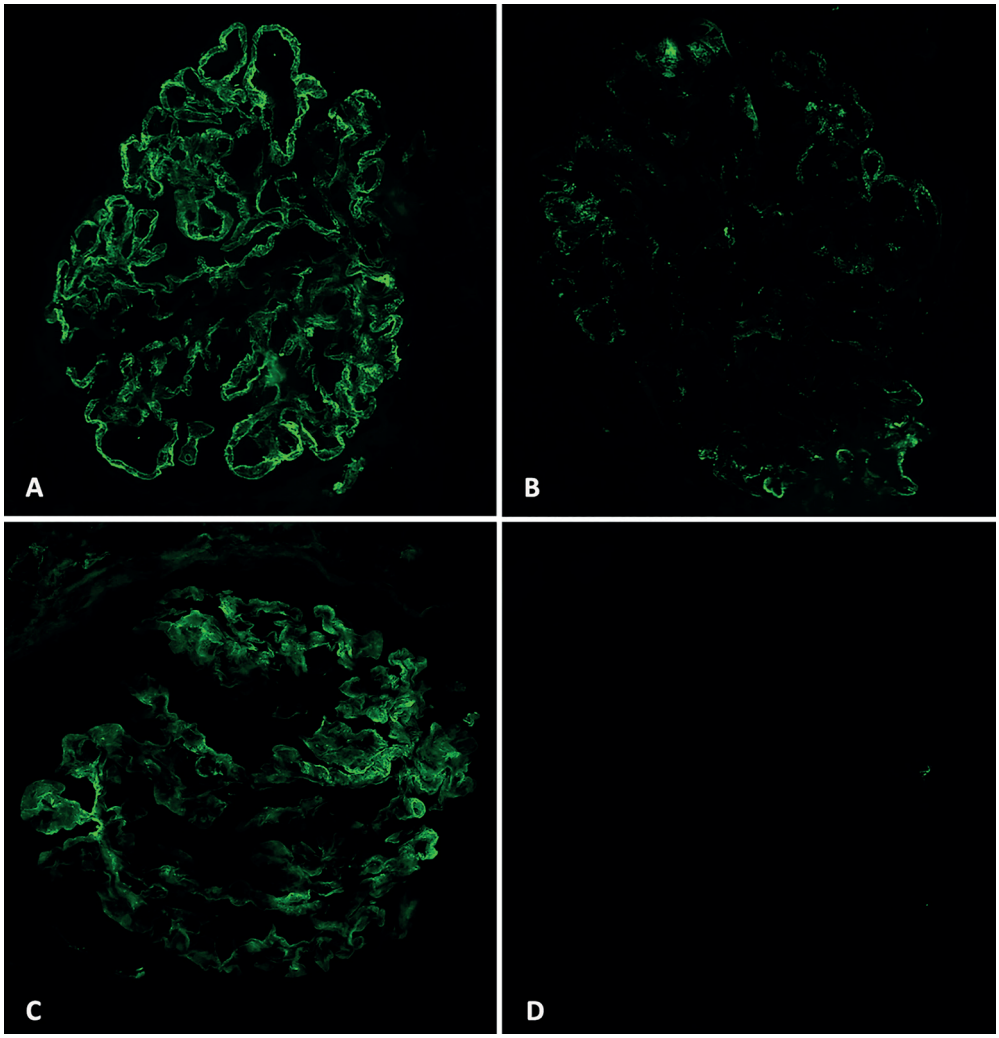

Figure 2. A, B: Second follow-up allograft biopsy from 2016 (very similar results were seen in the first biopsy from 2015). Staining for the complement degradation product C4d (A) demonstrates a strong pseudolinear global signal along glomerular capillary walls. In contrast, an IgM incubation (B) only shows a relatively weak, segmental, granular staining pattern. Of note: no significant glomerular staining is noted for IgG, IgA, C3, C1q, κ- and λ-light chains; C4d is not seen along peritubular capillaries (data not illustrated). C, D: Control case of native kidney with hereditary nephropathy/Alport syndrome: This native control biopsy with hereditary nephropathy shows segmental glomerular C4d deposits (C) along glomerular capillary walls resembling those illustrated in (A); significant glomerular IgM staining is not found (D). Confocal immunofluorescence microscopy; original magnification × 400.

1.1) and stable graft function (S-Cr: 0.45 mg/dL; eGFR 106 mL/min). Her blood pressure remained well controlled (< 95th percentile for age and height). Over the entire post-transplantation period, no donor-specific MHC-class I or class II antibodies were detected (tested 14 times; MFI/mean fluorescence intensity continuously < 200).

An attempt was made to follow-up on the performance of the contralateral donor kidney. Unfortunately, the organ was presumably placed with a recipient from outside of our organ-procuring organization, and data were not available.

Discussion

Proteinuria post renal transplantation can have different etiologies and often indicates a poor prognosis. In the current case, three differential diagnoses have to be considered: 1) donor-derived, transplanted hereditary nephropathy, 2) chronic rejection-induced transplant glomerulopathy, potentially due to antibody-mediated injury, and 3) a size-mismatch glomerulopathy.

Ad 1: Although ultrastructural glomerular changes bear similarities to "Alport syndrome", a donor-derived hereditary ne-

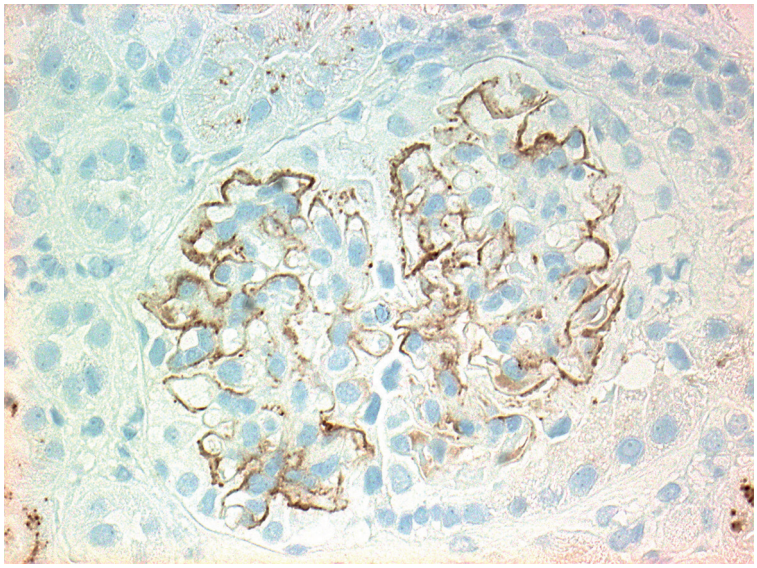

Figure 3. Second follow-up allograft biopsy from 2016 (very similar changes were seen in the first biopsy from 2015): Immunohistochemistry shows a pseudolinear staining pattern for C4d along glomerular capillary walls similar to findings illustrated in Figure 2A. Immunohistochemical incubation for C4d (Rabbit α-human polyclonal antibody, ALPCO) on formalin-fixed and paraffin-embedded tissue; original magnification × 400.

phropathy can be excluded from the list of differential diagnoses based on a normal staining pattern for collagen type IV α5 subunit as well as normal glomeruli by light, immunofluorescence, and electron microscopy in the implantation zero-hour biopsy. This set of findings supports the notion of a de novo glomerulopathy that developed post transplantation.

Ad 2: A diagnosis of rejection-induced "transplant glomerulopathy" can also be excluded with high probability. First and foremost, the glomerulopathy displays morphologic features not known to be rejection induced, i.e., substantial subepithelial and transmembranous capillary wall remodeling rather than subendothelial GBM duplication characterizing "transplant glomerulopathy" [5]. In addition, the lack of other signs of rejection further argues against a diagnosis of "transplant glomerulopathy", i.e., no concurrent peritubular capillaritis, no C4d staining along peritubular capillaries, no tubulointerstitial or vascular rejection, no transplant glomerulitis, and no multilamination of peritubular capillary basement membranes that is typical for chronic antibody-mediated rejection (ABMR) [6]. HLA donor-specific antibodies were not detected on repeat testing. Although we cannot totally exclude the remote possibility of injury caused by circulating non-HLA donor-specific antibodies, we think that the overall presentation pattern makes this differential diagnosis unlikely.

Ad 3: Based on the clinical scenario and morphology, the diagnosis of a "size-mismatch glomerulopathy" is most likely. Organ size mismatch, i.e., typically a too-small-for-body-size transplant as a cause for allograft dysfunction, proteinuria, and glomerular remodeling was first described by Truong et al. in 1991 [7]. Truong's observations were subsequently further characterized by Nadasdy et al. and Choung et al. [8, 9], reporting glomerulopathies in 9% of adult patients receiving donor kidneys from patients younger than 10 years of age. The authors described marked alterations of the glomerular filtration barrier very similar to the currently made observations. The previously-reported

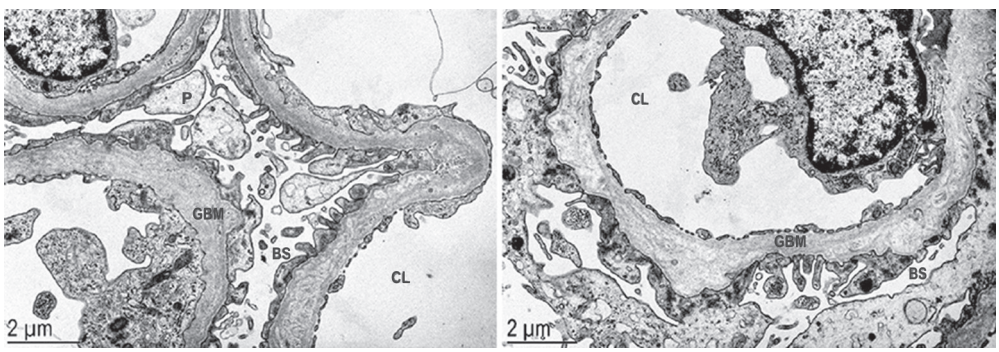

Figure 4. Electron microscopy of the 2015 and 2016 biopsies shows marked transmembranous remodeling of the glomerular capillary walls with basket-weaving of the lamina densa. Podocytes and endothelial cells are segmentally activated. There is no evidence of transplant glomerulopathy, i.e., no subendothelial duplication of glomerular basement membranes. CL = capillary lumen; GBM = glomerular basement membrane; BS = bowman's space; P = podocyte (transmission electron microscopy, uranyl acetate staining). Original magnification × 5,000, × 10,000.

patients presented with significant proteinuria and, in some instances, with "elevated" serum creatinine levels or graft failure [10, 11, 12, 13, 14, 15, 16, 17, 18]. Glomerular changes in these small donor organs transplanted into adult recipients were postulated to be the result of "extreme graft adaptation" in the setting of "high demand" and hyperfiltration. Findings in our case fit into the spectrum of "size-mismatch glomerulopathies", however, there is one major difference: our pediatric patient received a too-big-for-body-size donor kidney.

Pathophysiologic events governing capillary wall remodeling in the current case remain hypothetical, with transient glomerular hyperfiltration post transplantation as one potential causative event. We speculate that when an adult-size kidney is grafted into a small pediatric recipient, severe alterations of blood flow and intracapillary pressure inflict "stress" on the glomerular capillary walls and podocytes resulting in remodeling and changes to the filtration barrier. In our patient, likely protracted growth retardation with lack of "catch-up growth" generated a special "intraglomerular microenvironment" promoting GBM changes that were not reported in a recent series [19].

Our patient was followed for 64 months. During this period persistent proteinuria did not lead to chronic graft damage, and renal function remained stable suggesting a favorable mid-term prognosis. Whether long-term graft survival is affected remains to be determined.

Unusual (although not entirely unexpected) was the detection of isolated pseudolinear C4d staining along glomerular capillary walls not associated with C4d deposits along peritubular capillaries or diagnostic signs of ABMR. No other significant glomerular immunoglobulin or complement factor deposits were noted. Previous reports have illustrated that such isolated pseudolinear glomerular C4d staining is associated with structural capillary wall remodeling in native and transplant kidneys independent of the underlying triggering event and independent of antibody-mediated graft injury/rejection [20, 21, 22]. We interpret our current findings along the same line and view the isolated glomerular C4d accumulation as a reflection of the pronounced capillary wall restructuring – and not as a sign of antibody-induced injury. This notion is further supported by two other observations: 1) At the University of Basel in Switzerland, identical findings, including isolated glomerular C4d staining, were noted in a size-mismatch glomerulopathy post transplantation of a too-small-for-body size donor kidney lacking any sign of rejection [M.J. Mihatsch, H. Hopfer, personal communication]. 2) Cases of hereditary nephropathy/Alport syndrome in native kidneys that bear morphologic similarities to size-mismatch glomerulopathies can show pseudolinear glomerular C4d staining (Figure 2; unpublished personal observation). Whether complement activation via the lectin-pathway in glomerular capillary walls undergoing remodeling causes C4d deposits needs further evaluation in future studies.

In summary, this case describes for the first time "benign" nephrotic-range proteinuria due to a de-novo C4d-expressing glomerulopathy in a too-big-for-size mismatched kidney transplant. Follow-up demonstrated good allograft function over 64 months. Glomerular injury, capillary wall remodeling, and C4d accumulation are presumably caused by adaptive hemodynamic changes/stress including the activation of the lectin pathway. Since adult kidneys represent a crucial source of donor organs in pediatric patients [19], the current case provides further insight into possible complications encountered in severely size-mismatched renal allografts.

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Conflict of interest

The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or nonfinancial interest in the subject matter or materials discussed in this manuscript.

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