Effects of Octreotide-LAR Added-on Tolvaptan in Patients with ADPKD: Pilot, Randomized, Placebo-Controlled, Cross-Over Trial

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Supplemental Table 1. Number (%) of patients with concomitant medications at baseline in the study group considered as a whole (Overall) and according to the randomization to the two sequences of treatment with Octreotide-LAR followed by Placebo or with Placebo followed by Octreotide-LAR, both on top of Tolvaptan.

Supplemental Table 2. Total number of Serious AEs and Non-serious AEs throughout the whole study period (Overall) and during each period considered separately

SUPPLEMENTAL METHODS

MRI ACQUISITION - All MRIs were acquired at the ASST Papa Giovanni XXIII, Bergamo, Italy, on a 1.5T clinical scanner (Optima MR450wGEM; General Electrics Healthcare), using a Phased Array 32-channel Torso Body Coil, with no contrast media injection. MRI acquisition included morphological (T2-weighted, T1-weighted, and FIESTA), diffusion-weighted imaging, MR angiography, and phase-contrast (PC) sequences.

Coronal T2-weighted scans were acquired in breath-hold using a single-shot fast spin-echo sequence with fat saturation and 4mm slice thickness. Coronal T1-weighted scans were acquired in breath-hold using a 3D spoiled gradient interpolated (SPGR) sequence with 4 mm slice thickness and no fat saturation. In all anatomical sequences, cyst-free anterior and posterior slices were required to ensure full coverage of the kidneys.

Non-contrast enhanced MR angiography was acquired using a respiratory-triggered 3D fat saturated axial FIESTA sequence with inversion recovery pulses (Inhance 3D inflow IR). The sequence was reformatted to coronal plane to best evaluate abdominal vessel structure. Next, a breath-hold, cardiac-gated 2D PC velocity-sensitive sequence was acquired for left and right kidney artery separately, using the following parameters: field of view=320x272, matrix=256x256, slice thickness =7mm, TR/TE=6ms/3.5ms, 20° excitation flip angle, 62.5 Hz/pixel, 80 phases and 100 cm/s velocity encoding. The acquisition plane was set orthogonally to the kidney artery direction, approximately 1-2 cm far from the aorta, using the axial and coronal MR angiography as reference. In case of accessory kidney arteries, additional PC-MRI sequences were acquired.

MRI data were transferred in DICOM 16-bit format from the clinical scanner to digital media and sent to the Medical Imaging Laboratory, Istituto di Ricerche Farmacologiche Mario Negri IRCCS for quality control and image processing.

MRI PROCESSING - In the original version of the study protocol a preliminary estimate of kidney volume was planned by using the ellipsoid equation[1] and patients with estimated kidney volume <1000 ml or >2000 ml had to be excluded. However, the protocol was amended on July 6, 2020 in order to delete this restrictive exclusion criterion and the ellipsoid equation was not considered any longer.

Left and right kidneys were manually outlined on T1-weighted MR images by a trained operator (G.V.) blind to treatment, using ImageJ software (U. S. National Institutes of Health, Bethesda, Maryland, USA, https://imagej.nih.gov/ij/,[2] to accurately assess subtle volume changes.[3] Special attention was given to regions where kidneys and liver were adjacent. Kidney masks were created based on the outlines, and TKV was computed by multiplying the voxel count of the masks by voxel volume.

CKV was quantified from T2-weighted MR images using an in-house semi-automated method.[7] Before cyst volume quantification, the automated segmentation result was reviewed by an experienced operator (G.V.) and possibly manually edited. CKV was finally computed by multiplying the voxel count of the segmented image by voxel volume.

PC-MRI scans were visually inspected, and, in case of any artifact, were excluded. PC-MRI post-processing was performed by ImageJ software. Anatomical and corresponding velocity images were bi-linearly interpolated (4x4 pixels) and cropped to a squared region centred on the kidney artery. Rigid body registration was then performed on the anatomical images, and the corresponding velocity images were co-registered, using the MultiStackReg plugin. Kidney artery outline was drawn on the registered anatomical image with highest definition, including the whole signal gradient, and was kept constant on the time series. Kidney artery mask was finally computed from the outline. For each kidney artery, velocity and flow were computed by in-house software written in Matlab version 2018a, based on velocity images. The average velocity in the kidney artery mask, expressed in cm/s, was computed, and multiplied by the kidney artery mask area by 60 to obtain the renal artery blood flow (RABF), expressed in ml/min. RABF values were finally averaged over all phases, and individual RABFs were summed up to have the total renal blood flow (RBF). RPF, expressed in ml/min, was computed as RBF * [(1 – hematocrit)/100], and FF, expressed in percentage, was computed as GFR/RPF * 100. RVR was calculated as the ratio between mean arterial pressure and mean RBF.

INCLUSION CRITERIA

Adult (>18-yr-old) men and women who had signed informed consent and with a clinical and ultrasonographic diagnosis of ADPKD; serum creatinine < 1.2 mg/dl (for man) and < 1.0 mg/dl (for woman) and changes <30% over the last year; creatinine clearance ≥80 ml/min/1.73m2 measured one to two weeks apart: one during the prescreening or during the screening period (alternatively, two measurements during the screening period, one to two weeks apart) and the other at baseline; female participants must be of non-childbearing potential or must agree to abstinence or use a highly effective form of contraception.

EXCLUSION CRITERIA

Pregnant or lactating women and women with childbearing potential who did not agree to abstinence or to use of a highly effective form of contraception were excluded along with patients with concomitant systemic, kidney parenchymal or urinary tract disease; diabetes; urinary protein excretion rate >300 mg/24 hours and/or abnormal urinalysis suggestive of concomitant glomerular disease; symptomatic urinary and/or biliary tract infection or obstruction; hemorrhagic or complicated cysts which might acutely affect kidney function and volumes; QT-related electrocardiography abnormalities; cancer and major systemic diseases that could prevent completion of the planned follow-up or interfere with data collection or interpretation; known hypersensitivity to the investigational medical active substances or to any of the excipients or to benzazepine or benzazepine derivatives; elevated liver enzymes and/or signs or symptoms of liver injury prior to initiation of treatment that meet the requirements for permanent discontinuation of Tolvaptan; anuria, volume depletion or hypernatremia; failure to perceive or respond to thirst; ferro-magnetic prosthesis, aneurysm clips, severe claustrophobia or any other contraindication to MRI evaluation; psychiatric disorders and any condition that could prevent full comprehension of the purposes and risks of the study; participation in another interventional clinical trial within the 4 weeks prior to screening.

RANDOMIZATION - The two treatment sequences were centrally allocated by an independent investigator (G.A.G.) who was not directly involved in conducting the study. The web-based, computer-generated randomization list was created using SAS (version 9.2). Investigators involved in data handling and analyses were blinded to treatment allocation. A blinded-to-treatment adjudicating group reviewed to evaluate safety.

SAMPLE SIZE ESTIMATION - At the end of the 4-week treatment period, TKV (secondary efficacy outcome) was predicted to decrease by 5% (from 1364 to 1286 ml) with Tolvaptan plus placebo combination therapy and by 10% (from 1364 to 1228 ml) by Tolvaptan and Octreotide-LAR combination therapy as compared to pretreatment values. With this assumption, end considering the pilot nature of the study, the 15 patients - that were all exposed to both treatment periods - planned to complete the study provided an adequate power to detect a treatment effect on TKV.

SUPPLEMENTAL RESULTS

PRIMARY AND CO-PRIMARY EFFICACY VARIABLES

Changes in 1-month GFR. GFR changes during the Tolvaptan and placebo or Tolvaptan and Octreotide-LAR treatment periods significantly differed from GFR changes during the Run-in period (p= 0.002 and p=0.007, respectively). Conversely, the GFR non-significantly increased by 4 (-2 to 8) ml/min/1.73m^2 during the 1-month wash-out period from Tolvaptan and placebo treatment and by 5 (0 to 9) ml/min/1.73m^2 during the 1-month wash-out period from Tolvaptan and Octreotide-LAR treatment (Figure 1, Upper Panel). GFR changes during the two wash-out periods significantly differed from GFR changes during the corresponding treatment periods with Tolvaptan and placebo (p=0.002) or with Tolvaptan and Octreotide-LAR (p=0.001).

Changes in 1-week GFRAs for 1-month GFR changes, the GFR reductions observed after 1-week treatment with Tolvaptan and placebo (p= 0.02) or with Tolvaptan and Octreotide-LAR add-on therapy (p<0.001) significantly differed from the (opposite) GFR changes observed during the corresponding wash-out periods (Figure 1, Lower Panel).

SECONDARY EFFICACY VARIABLES

Total Kidney Volume - TKV significantly increased by 31 (7 to 52) (p=0.002) and by 30 (17 to 53) ml (p=0.001) during the Tolvaptan and placebo and the Tolvaptan and Octreotide-LAR wash-out periods, respectively (Figure 2, Upper Panel). TKV changes during the two wash-out periods significantly differed from TKV changes during the corresponding treatment periods with Tolvaptan and placebo (p=0.01) or with Tolvaptan and Octreotide-LAR combination therapy (p<0.001). Similar results were obtained by measuring height-adjusted TKV (data not shown).

Cystic Kidney Volume - Cystic Kidney Volume non-significantly increased by 10 (-27 to 21) ml during the 1-month Tolvaptan and placebo treatment period (p=0.49) whereas it decreased significantly by 22 (9 to 47) ml during the 1-month Tolvaptan and Octreotide-LAR treatment period (p=0.01). Cystic Kidney Volumes changes during the two treatment periods non-significantly (p=0.07) differed by 25 (-3 to 50) ml. Cystic Kidney Volumes non-significantly increased by 11 (-14 to 48) ml (p=0.83) during the wash-out period from Tolvaptan and placebo whereas it significantly increased by 20 (9 to 42) ml (p=0.009) during the wash-out period from Tolvaptan and Octreotide-LAR (Figure 2, Lower Panel). Cystic Kidney Volumes changes during

the wash-out period from Tolvaptan and placebo and the corresponding treatment period did not differ significantly (p=0.844), whereas Cystic Kidney Volumes changes during the wash-out period from Tolvaptan and Octreotide-LAR and the corresponding treatment period were significantly different (p=0.001)

OTHER EXPLORATORY EFFICACY VARIABLES

Systemic hemodynamics **-** Heart rate changes during the Tolvaptan and Octreotide-LAR treatment period significantly differed (p=0.02) also from changes during the Run-in period (Figure 3, Lower Left Panel). Notably, albumin fractional clearance decreased during the Tolvaptan and Octreotide-LAR treatment period whereas it non-significantly increased during the Tolvaptan and placebo treatment period (Figure 3, Lower Right Panel). These two opposite changes were significantly different (p=0.008). Changes during the Tolvaptan and Octreotide-LAR treatment period significantly differed also by the opposite changes observed during the Run-in period (p=0.01).

Functional Parameters **-** Consistently with the concomitant changes in GFR, RBF non-significantly decreased during the Tolvaptan and placebo (p=0.07) and the Tolvaptan and Octreotide-LAR (p=0.10) treatment periods as compared to the Run-in period. RPF also non-significantly decreased during the Tolvaptan and placebo treatment period (p=0.10) as compared to the Run-in period. This change was statistically significant during the Tolvaptan and Octreotide-LAR add-on therapy (p=0.04). RVR decreased significantly during the wash-out period from Tolvaptan and placebo ($p<0.05$) whereas FF decreased significantly during the Tolvaptan and placebo treatment period ($p<0.05$). Other changes were not significant.

SERIOUS AND NON-SERIOUS ADVERSE EVENTS - During the Tolvaptan and Octreotide-LAR treatment period and/or during the Tolvaptan and placebo treatment period all patients reported in various combinations symptoms related to the diuretic effect of Tolvaptan such as polyuria with or without nocturia, increased thirst and dry mouth (Table 3 and Supplemental Table 2). Gastrointestinal symptoms related to Octreotide-LAR were also common including diarrhea, a-colic stools, abdominal pain or distension and nausea or vomiting (Table 3 and Supplemental Table 2). However, serious adverse events related to either Tolvaptan and to Octreotide-LAR were observed in only one patient during the first treatment period with Tolvaptan and Octreotide-LAR. This patient permanently discontinued both medications, but was maintained into the study and completed all study visits. In two patients the Tolvaptan dose was reduced with symptoms relief. Four

patients reported pain at the site of Octreotide-LAR injection. Other non-serious adverse events were usually mild in intensity and self-limited and never required treatment interruption.

LEGENDS TO THE SUPPLEMENTAL FIGURES

Supplemental Figure 1. Study design

▲Creatinine Clearance, Kidney and Liver ultrasound evaluation: ▌Total (TKV) and cystic (CKV) kidney volumes and kidney function parameters assessed by magnetic resonance imaging (MRI) and/or calculated by standard formulas, glomerular filtration rate (GFR) measured by the Iohexol plasma clearance technique and routine laboratory evaluations; ●GFR and routine laboratory evaluations

Supplemental Figure 2. Study flow chart

Overall, 75 potentially eligible patients were identified from the population of patients afferent to the ADPKD Outpatient Clinic of the Unit of Nephrology of the ASST Papa Giovanni XXIII and were referred to the Clinical Research Center. The study protocol and all its implications were discussed with all referred patients: 41 denied their consent whereas 34 signed the written informed consent to study participation. Of these 34 patients, four subsequently withdrew their consent, one was lost to follow-up and seven were found not to full-fill all the selection criteria of the study. Thus, between 22 January, 2019 and 2 July, 2021, 22 patients entered the Run-in phase of the study. One of these patients was withdrawn during the Run-in because of an episode of colic pain due to nephrolithiasis. Another patient was withdrawn at completion of the Run-in because at baseline evaluation he was found to have creatinine clearance < 80/ml/min/1.73m^2. Thus 20 patients were randomized: ten to each treatment sequence. However, immediately after randomization, one patient allocated to the sequence Tolvaptan and Octreotide-LAR followed by Tolvaptan and placebo combination therapy was lost to follow up. The patient never assumed any of the study drugs. Thus, 19 patients entered the two treatment sequences with Tolvaptan- Placebo followed by Tolvaptan-Octreotide-LAR (n=10) or with Tolvaptan-Octreotide-LAR followed by Tolvaptan-Placebo (n=9). During the wash out period of the first treatment sequence one patient was withdrawn because of an adverse event (SARS-CoV-2 infection followed by a left bundle branch block). Thus, 18 patients (9 per treatment sequence) completed the whole study period and were available for the analyses.

Supplemental Table 1. Number (%) of patients with concomitant medications at baseline in the study group considered as a whole (Overall) and according to the randomization to the two sequences of treatment with Octreotide-LAR followed by Placebo or with Placebo followed by Octreotide-LAR, both on top of Tolvaptan.

	Overall n=19	**Octreotide-LAR-Placebo** n=9	**Placebo-Octreotide-LAR** n=10	**P Value**
Antihypertensive agents				
Any	14 (74)	6 (60)	8 (89)	0.629
Calcium-channel blockers	13 (68)	5 (50)	8 (89)	0.350
Angiotensin-converting-enzyme (ACE) inhibitors	9 (47)	4 (40)	5 (56)	1.000
Sympatholytic agents	6 (32)	2 (20)	4 (44)	0.629
Beta blockers	2 (11)	1 (10)	1 (11)	1.000
Angiotensin receptor blockers (ARBs)	1 (5)	0	1 (11)	1.000
Diuretics	1 (5)	0	1 (11)	1.000
Lipid-lowering agents				
Any	1 (5)	0	1 (11)	1.000
Statins	1 (5)	0	1 (11)	1.000
Metabolic agents				
Any	4 (21)	3 (30)	1 (11)	0.303
Vitamin D3 (cholecalciferol)	3 (16)	2 (20)	1 (11)	0.582
Allopurinol	2 (11)	2 (20)	0	1.000

Data are numbers and percentages (round brackets). Fisher's Exact test.

Supplemental Table 2. Total number of Serious AEs and Non-serious AEs throughout the whole study period (Overall) and during each period considered separately

Adverse Events	Overall *(N=19)*	Run-in *(N=19)*	Tolvaptan-placebo *(N=19)*	WO Tolvaptan-placebo *(N=19)*	Tolvaptan-Octreotide-LAR *(N=18)*	WO Tolvaptan-Octreotide-LAR *(N=18)*
Any adverse event	**238**	**35**	**81**	**18**	**93**	**11**
Total serious AEs	3	0	0	1	2	0
Covid-19	1	0	0	1	0	0
Elevated transaminase levels	1	0	0	0	1	0
Elevated CPK levels	1	0	0	0	1	0
Total non-serious AEs	235	35	81	17	91	11
Left bundle branch block*	1	0	0	1	0	0
Polyuria†	35	0	17	0	18	0
Increased thirst†	35	0	22	0	18	0
Nocturia†	14	0	6	0	8	0
Flu like syndrome†	13	5	5	3	0	0
Diarrhea, loose stools†	9	0	1	0	7	1
Abdominal pain/distension†	11	0	2	3	5	1
Dry mouth†	9	0	5	0	4	0
Light-coloured stools	7	0	1	0	6	0
Tendon tear, joint or traumatic pain†	11	6	2	3	0	0
Nausea/dyspepsia	6	1	2	0	3	0
Back pain/sciatica	6	2	0	0	0	4
Constipation/borborisms	5	0	3	1	1	0
Anckle edema	4	4	0	0	0	0
Headache	4	1	2	1	0	0
Fatigue	4	0	1	0	3	0
Site-injection pain	4	0	0	0	4	0
Arterial Hypertension	4	3	1	0	0	0
Anemia, leukopenia or neutropenia†	5	1	3	1	0	0
Dyslipidemia	3	2	1	0	0	0
Elevated CPK levels	3	1	1	0	1	0
Urinary tract infection/dysuria	3	1	0	0	0	2
Arm pain due to SARS-CoV-2 vac.†	3	1	1	1	0	0
Acute urticaria/itching	3	0	1	0	2	0
Herpes labialis	2	1	0	0	1	0
Elevated serum uric acid levels	2	0	0	0	2	0
Covid-19	2	0	0	0	0	2
Breast nodule, breast cyst†	2	0	1	1	0	0
Palpitations/extrasystoles†	2	0	0	1	1	0
Left side numbness/tingling†	2	0	0	0	2	0
Pancreatic cyst	1	1	0	0	0	0
Kidney stones	1	1	0	0	0	0
Leg cramps	1	1	0	0	0	0
Hypokalemia	1	1	0	0	0	0
Sinusitis	1	1	0	0	0	0
Testicular pain	1	1	0	0	0	0
Dyspnea	1	0	1	0	0	0
Elevated LDH levels	1	0	1	0	0	0
Elevated AST levels	1	0	1	0	0	0
Menorrhagia	1	0	1	0	0	0
Microalbuminuria	1	0	1	0	0	0
Post COVID-19 vacc. syndrome	1	0	1	0	0	0

Keratoconjuntivitis	1	0	1	0	0	0
Abdominal hematoma	1	0	1	0	0	0
Hypertensive retinopathy grade 1	1	0	0	1	0	0
Flatulence	1	0	0	0	1	0
Vomiting	1	0	0	0	1	0
Elevated serum creatinine levels	1	0	0	0	1	0
Suspected hypoglycemia	1	0	0	0	1	0
Facial numbness	1	0	0	0	1	0

*Discontinuation of trial due to AE. Same events may occure during different treatment periods.

SUPPLEMENTAL REFERENCES

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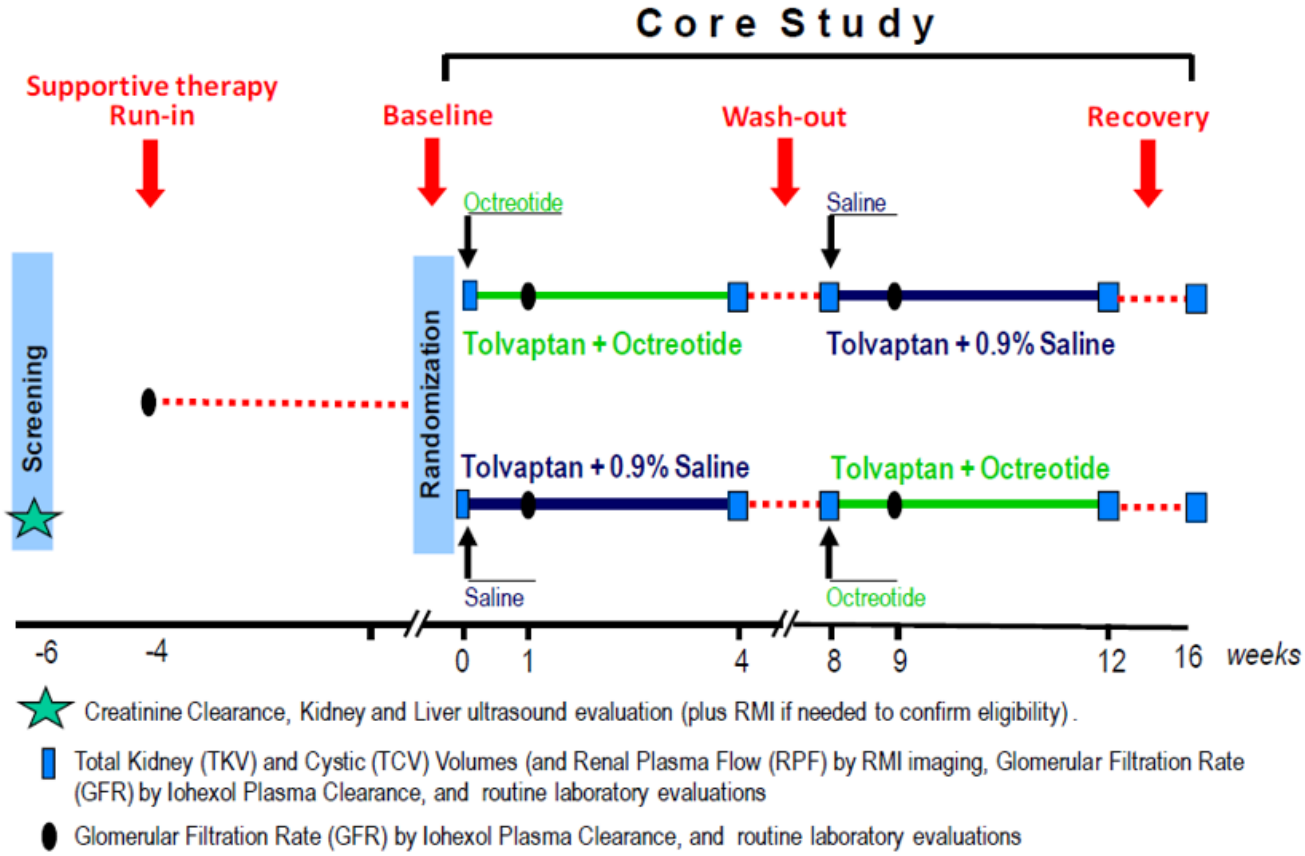
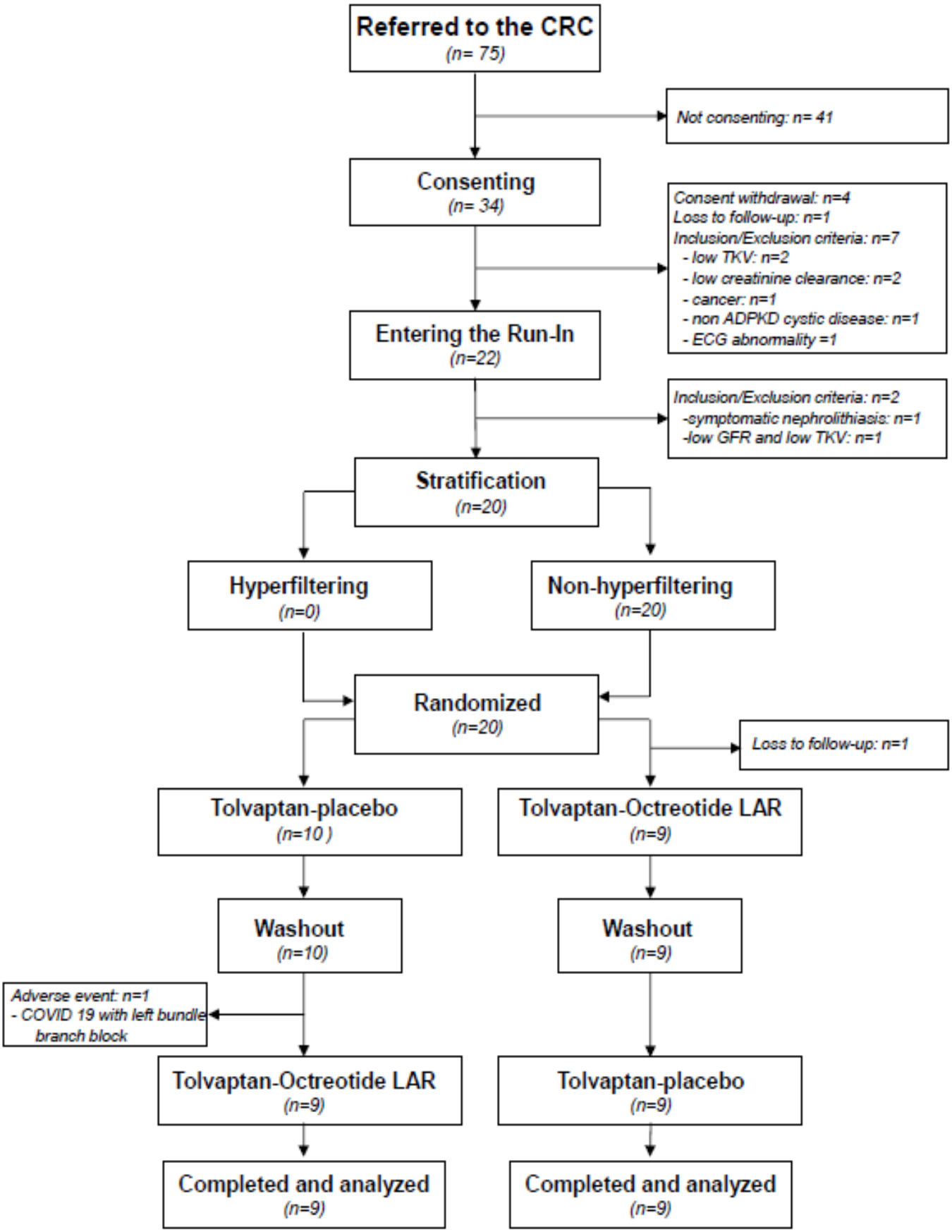


Supplementary Figure1



Supplementary Figure 2