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Renal involvement in sarcoidosis: histological patterns and prognosis, an Italian survey

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Abstract. Background: Granulomatous interstitial nephritis in sarcoidosis (sGIN) is generally clinically silent, but in <1% causes acute kidney injury (AKI). **Methods:** This Italian multicentric retrospective study included 39 sarcoidosis-patients with renal involvement at renal biopsy: 31 sGIN-AKI, 5 with other patterns (No-sGIN-AKI), 3 with nephrotic proteinuria. We investigate the predictive value of clinical features, laboratory, radiological parameters and histological patterns regarding steroid response. Primary endpoint: incident chronic kidney disease (CKD) beyond the 1°follow-up (FU) year; secondary endpoint: response at 1°line steroid therapy; combined endpoint: the association of initial steroid response and outcome at the end of FU. **Results:**

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Complete recovery in all 5 No-sGIN-AKI-patients, only in 45% (13/29) sGIN-AKI-patients (p=0.046) (one lost in follow-up, for another not available renal function after steroids). Nobody had not response. Primary endpoint of 22 sGIN-AKI subjects: 65% (13/20) starting with normal renal function developed CKD (2/22 had basal CKD; median FU 77 months, 15–300). Combined endpoint: 29% (6/21) had complete recovery and final normal renal function (one with renal relapse), 48% (10/21) had partial recovery and final CKD (3 with renal relapse, of whom one with basal CKD) (p=0.024). Acute onset and hypercalcaemia were associated to milder AKI and better recovery than subacute onset and patients without hypercalcaemia, women had better endpoints than men. Giant cells, severe interstitial infiltrate and interstitial fibrosis seemed negative predictors in terms of endpoints. **Conclusions:** sGIN-AKI-patients with no complete recovery at 1°line steroid should be treated with other immunosuppressive to avoid CKD, in particular if males with subacute onset and III stage-not hypercalcaemic AKI.

Keywords: Renal sarcoidosis, sGIN, AKI, sACE, Hypercalcaemia, Giant cells, Steroids, Acute onset, Subacute onset, GenPhenReSa phenotypes, Interstitial infiltrate, Interstitial fibrosis

Introduction

Sarcoidosis is an idiopathic multisystemic granulomatous disorder, histologically charaterized by epithelioid non-caseating granulomas which involves primarily lungs, mediastinum, lymph nodes, liver, eyes, skin. All organs could be affected by disease, that rarely strikes kidneys, central nervous system and heart (1). In susceptible hosts an abnormal T-helper 1 response to an unknown not degradable antigen stimulates macrophages and dendritic cells to transform into epithelioid cells and to fuse together into multinucleate giant cells. The nodular inflammatory infiltrate characterizing sarcoid granulomatous interstitial nephritis (sGIN) includes one or more distinct aggregates of epithelioid cells with or without multinucleate giant cells (2) (Figure 1).

There isn't an accurate datum of prevalence of sarcoidosis in the world, varying according to case studies from 4.7 to 64 cases per 100,000 individuals with an incidence from 1.0 to 35.5 per 100,000 individuals per year (3). Prevalence and incidence depend on different age, sex, ethnicity and geographic origin. The highest rates are reported in Northern Europe and in African-American individuals, the lowest rates in Asia (4). The Italian Register on Diffuse Infiltrative Lung Disorders reported a frequency of 1,063 subjects among 79 Italian centers adhering to the project (5). In last years interest for sarcoidosis is increased in Italy: for instance in 2017 Beghè et coll investigated prevalence and spatial distribution of cases and environmental exposures associated with sarcoidosis in greater Parma, a North-Italy area composed in 47 Municipal Districts (3447.4 km2, population 431,000) (6). 223 patients were identified (58.3% female, 41.7% male, average age 50.6±15.4 years). The study revealed a high prevalence of sarcoidosis in greater Parma area: 49 per 100,000 individuals (the definition for rare disease according to the European Commission on Public Health is prevalence <50 cases in 100,000 people) (7). This study was not sufficient to establish a clear correlation between the onset of sarcoidosis and environmental risk factors. Considering patients with acute or chronic sarcoidosis, renal involvement is not fre-

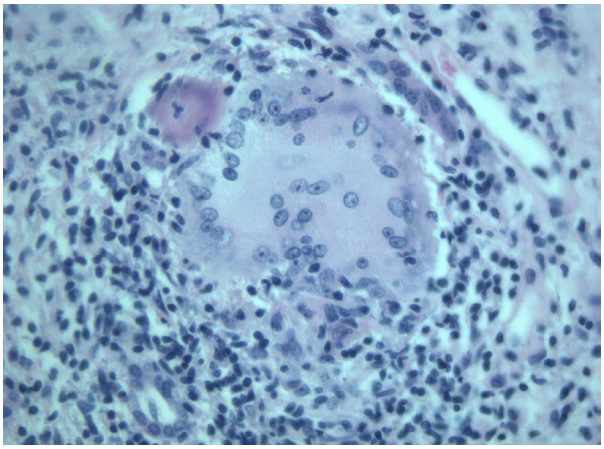

Figure 1. A multinucleate giant cell in sGIN, contributed by Dr Irene Acquistapace.

quent, as it emerges from geo-epidemiological big data approach to sarcoidosis: in 2019 a review collected large series (>100 patients) of sarcoidosis reported in the PubMed library in the last 20 years, renal involvement frequency was observed in 0.3% patients in Northern Europeans (6/2,209 patients with exthratoracic sarcoidosis (ET-S), total patients 71,566), 1.8% patients in Southern European cohorts (26/1,477 ET-S patients, total patients 5,902), 1.1% patients in US cohorts (28/2,436 ET-S patients, total patients 7,263), 3.5% patients in Japanese cohorts (36/1,027 ET-S patients, total patients 3,315) (8).

Genotype–Phenotype Relationship in Sarcoidosis (GenPhenReSa) project is an European multicentre study designed to investigate the influence of genotypes on disease phenotypes and comprising 2,163 Caucasian patients who were phenotyped at 31 study centres according to a standardised protocol (9). In GenPhenReSa project patients had this organ involvement: 92.6% (n=1,664) lungs, 77.0% (n=1,355) mediastinal and/or hilar lymph nodes, 16.1% (n=342) skin, 7.8% (n=163) eyes, 7.5% (n=158) joints, 3.1% (n=67) kidneys. At chest radiography, 16.4% (n=347) had no lung sarcoidosis (Scadding type 0), 3.9% (n=83) had lung fibrosis (Scadding type IV).

The incidence and prevalence of sarcoid granulomatous interstitial nephritis (sGIN) on course of sarcoidosis isn't known. Generally it's a silent finding observed at autopsy in 7-23% of sarcoidosis patients (10). sGIN is mainly observed at renal biopsies (RBx) performed in case of acute kidney injury (AKI), occuring in <1% of sarcoidosis patients (11). This explains why renal sarcoidosis has a low frequency considering American monocentric cohorts of about 10,000 native kidney biopsies performed in 10 year-period: from 0.1% (11/9,779) at Harvard Medical School (12) to 0.18% (19/10,023) at Johns Hopkins University (13). Despite its low frequency sGIN deserves a particular attention, often having a not benign prognosis (14) and progressing to chronic kidney disease (CKD) and end stage kidney disease in some patients (13, 15). This multicentric retrospective study was carried out in Italy to collect the widest possible case series of biopsies with renal sarcoidosis diagnosis. We analysed the role of RBx in supporting the laboratory, radiological and clinical findings to rule out the diagnosis and investigate if histological patterns could have predictive value in respect of steroid therapy.

Methods

From 2013 to 2019 we performed a survey in many Italian Nephrologies with support of Immunopathology Group of Italian Society of Nephrology.

In Milan area: Bassini; San Carlo; Niguarda; Sacco; San Raffaele. Other centres in Lombardia: Sant'Anna, Como; Papa Giovanni XXIII, Bergamo; Spedali Civili, Brescia; Sondrio Hospital; Istituti Ospitalieri, Cremona. In Piemont: San Giovanni Bosco, Torino; San Luigi, Orbassano; Rivoli Hospital. In Veneto: Santa Maria dei Battuti, Treviso; Santa Maria del Prato, Feltre. In Trentino Alto Adige: Santa Chiara, Trento. In Emilia Romagna: Policlinico Sant'Orsola-Malpighi, Bologna. In Umbria: Santa Maria della Misericordia, Perugia. In Lazio: Belcolle, Viterbo. In Puglia: Policlinico di Bari. In Sicily: Policlinico Gaetano Martino, Messina. Beyonds Nephrologies, these units participated to the study: Pneumology of Santa Maria alle Scotte, Siena (Regional Referral Centre for Sarcoidosis); Nephropathology Centre in San Gerardo, Monza; Pathologies of Niguarda and of Sacco. All data were anonymized and pooled for a retrospective analysis.

Every centre received a questionnaire to collect full clinical history, comprehensive drug history, clinical and histological findings, abdominal ultrasonography scan, laboratory analysis as well as follow-up data. Lab exams included blood count, serum creatinine (sCr), 25-hydroxyvitamin D [25(OH)D], 1-25-hydroxyvitamin D [1-25(OH)D], parathyroid hormone (PTH), serum angiotensin converting enzyme (sACE), urine sediment, 24h-proteinuria (24h-uProt), 24h-calciuria. Hypercalcaemia was defined as calcaemia >10.5 mg/dl or ionized calcium >1.29 mmol/L, hypercalciuria as calciuria >300 mg/24h, hyperphosforemia as phosforemia >5.0 mg/dl, hyperphosphaturia as phosphaturia >1300 mg/24h, high level of Vitamin D if 25(OH)D >100 ng/ml and/or 1-25(OH)D >55 pg/ml. Microhematuria and leukocyturia were respectively defined as a red blood cell count of ≥5 per high-power field and a white blood cell count of ≥5 per high-power field. sACE levels were considered to have increased if above the local threshold. Proteinuria was defined as urinary protein excretion of more than 150 mg per

day (10 to 20 mg per dL), nephrotic proteinuria as urinary protein >3g/24h. Nephrotic syndrome was defined by nephrotic proteinuria, hypoalbuminemia (albumin < 3g/24h), dyslipidemia and fluid retention with severe swelling (16).

Sarcoidosis diagnosis was based on histological pattern, radiologic features, and compatible clinical presentation with exclusion of other causes of granulomatous inflammation. Renal sarcoidosis was carefully verified, with the presence of renal interstitial non-caseating granuloma without evidence of acid-fast bacilli or fungi at RBx.

Inclusion criteria: RBx documenting sarcoid involvement according to one of these histological settings:

1. histological renal finding consisting with renal sarcoidosis: GIN, nephrocalcinosis.
2. other renal pattern together with extra-renal not-caseating granulomas (biopsy proved).
3. other renal pattern adding on asymptomatic bihilar lymphadenopathy.

Absence of non-caseating granulomas in renal tissue wasn't an exclusion criteria if associated with other organs involved by ascertained sarcoidosis.

Exclusion criteria: presence of other causes of granulomatous inflammation: tuberculosis and mycobacterial infections, fungal infections, leprosy, brucellosis, berylliosis and other pneumoconiosis, hypersensitivity pneumonia, Crohn's disease, vasculitis (granulomatosis with polyangiitis and eosinophilic granulomatosis with polyangiitis), granulomatous reaction due to neoplasm (in particular breast cancer, lung cancer, Hodgkin lymphoma), foreign-body reaction (heroin, cholesterol atheroembolism), drug-induced interstitial nephritis (non-steroidal anti-inflammatory drugs, allopurinol, fluoroquinolone antibiotics, diuretics, HIV-antiretroviral, TNFαinhibitors, αinterferon), crystals nephropathies.

Histological analysis: All 39 renal samples were examined through light microscopy (staining with haematoxylin and eosin, periodic acid-Schiff reaction, silver methenamine and trichrome), 18 with immunofluorescence microscopy (antibodies to IgA, IgG, IgM, C1q, C3, C4 routinely used). Congo-Red staining was performed in 12 cases and Ziehl-Neelsen staining in 2 cases. Mycobacteria infection was even excluded with Quantiferon test. Table 1 shows the case profiles, as follows: sex, ethnicity, blood arterial hypertension (HTA), mellitus diabetes (MD), cardiovascular disease (CAD), vasculopathy (VASC), chronic kidney disease (CKD), multiple myeloma (MM).

In order to be more confident as possible with renal sarcoidosis, we verified these 6 aspects:

1. The presence of noncaseating granulomas on biopsy of almost one other organ apart from kidney (ExtraRenal Granulomatosis)
2. known sarcoidosis diagnosed before RBx (S. before RBx)
3. systemic involvement of disease when RBx was performed (systemic S. at RBx)
4. hypercalcaemia and hypercalciuria (Calcium anomalies)
5. recurrence of sarcoidosis after RBx (S. relapses)
6. sarcoidosis family history.

We consider recurrent disease any disease flare that necessitated a change in therapy.

Endpoints: Primary endpoint: incident CKD beyond the first follow-up (FU) year. Secondary endpoint: response at first line steroid therapy as complete recovery (CR), partial recovery (PR) or not response (NR) through a comparison between basal estimated glomerular filtration rate (eGFR) before AKI episode and maximum eGFR under steroid therapy: for CR this difference is < 10 ml/min, for PR is > 10 ml/min with Max GFR ≥ 25% of basal eGFR; for NR if Max GFR < 25% of basal eGFR. Combined endpoint: the association of initial steroid response and outcome at the end of FU (considered for sGIN-AKI patients). The evaluated glomerular filtration rate (eGFR) was calculated according to the CKD-EPI creatinine 2009 equation. CKD was defined according to KDIGO 2012 as eGFR <60ml/min present for > 3 months (17). FU was investigated until September 2019. For primary outcome analysis we excluded patients if last FU seric creatinine (sCr) was within one year from RBx, to be sure of CKD. In primary endpoint, we performed 2 analysis: one considering as outcome the incident CKD, the other considering a relevant renal function (RF) worsening, defined as eGFR decrease below 60 ml/min or delta GFR >20ml/min (difference between basal eGFR before RBx and final eGFR at last FU)

Table 1. Population

Pt ID (n=39)	Sex	Ethn.	HTA	MD	CAD	VASC	CKD	MM	ExtraRenal Gran.	S. before RBx	systemic S. at RBx	Calcium anomalies	S. relapses	S. family history
sGIN-AKI														
1	0	0	1	0	0	0	0	0	1	1	1	0	0	0
2	1	0	0	0	0	0	0	0	0	0	1	0	0	0
3	1	0	0	0	0	0	0	0	0	0	1	0	0	0
4	1	1	0	0	0	0	0	0	0	0	1	0	0	0
5	1	0	1	0	0	0	1	0	0	0	1	0	1	0
6	1	1	1	0	0	0	0	0	0	0	1	0	0	0
7	1	0	0	0	0	1	0	0	1	1	1	0	0	0
8	1	0	1	0	0	0	0	0	0	1	1	1	1	1
9	0	0	0	0	0	0	0	0	0	0	1	0	0	0
10	1	0	0	0	0	0	0	0	0	0	0	0	0	0
11	1	0	0	0	0	0	0	0	0	0	1	1	1	0
12	1	0	1	0	0	0	0	0	1	0	1	0	1	0
13	1	0	1	0	0	0	0	0	0	0	1	1	1	0
14	1	0	1	0	0	0	0	0	1	1	1	0	0	0
15	1	0	1	0	1	0	0	0	0	1	1	0	0	0
16	1	0	1	0	0	0	0	0	0	0	1	1	1	0
17	1	0	1	1	0	0	0	0	0	0	1	0	0	0
18	1	0	0	0	0	0	0	0	0	0	0	1	0	0
19	0	0	0	0	1	0	0	0	0	0	1	0	0	0
20	1	0	1	1	0	0	0	0	0	0	0	0	1	0
21	1	0	1	1	0	0	0	0	1	1	1	0	0	0
22	1	0	1	0	0	0	0	0	0	0	1	0	1	0
23	1	0	1	1	0	0	0	0	0	0	1	1	0	0
24	1	0	0	0	0	0	0	0	0	0	1	0	0	0
25	1	0	1	0	0	0	0	0	0	1	1	0	0	0
26	1	0	1	0	0	0	1	0	0	0	0	0	0	0
27	1	0	1	0	0	0	0	1	0	0	0	1	0	0
28	1	0	0	0	0	0	0	0	0	0	1	0	0	0
29	1	0	1	1	0	0	0	0	0	0	1	1	1	0
30	0	0					0	0	0	0	1	0	0	0
31	0	0	0	0	0	0	0	0	1	1	1	0		0
No-sGIN-AKI														
32	0	0	0	0	0	0	1	0	0	0	1	1	0	0
33	1	0	0	0	0	1	0	0	1	1	1	1	0	0
34	0	0	0	0	0	0	0	0	0	0	1	1	0	0
35	0	1	0	0	0	0	0	0	0	0	1	0	0	0
36	0	0	0	0	0	0	1	0	1	0	1	1	0	0
Nephrotic Syndrome														
37	0	0	0	0	0	0		0	1	0	1	1	0	0
38	0	0	1	0	0	0		0	0	0	1	0	1	0
39	1	0	1	0	0	0		0	0	1	1	0	0	0

with final eGFR >60 ml/min. We considered 2 combined outcomes: association of steroid response and final normal RF/final CKD, association of steroid response and final RF worsening/not RF worsening. Patients with nephrotic proteinuria didn't have renal failure and weren't included in the outcome analysis. No patient took immunosuppressant drugs in combination with steroids as 1° line therapy. AKI definition staged for severity according to the latest classification proposed by the Acute Kidney Injury Working Group of KDIGO (18):

- Stage 1: sCr increase ≥ 1.5–1.9 fold from baseline OR absolute increase in sCr ≥0.3 mg/dL;
- Stage 2: sCr increase ≥2.0–2.9 fold from baseline;
- Stage 3: sCr increase ≥3.0 fold from baseline OR sCr increase ≥4.0 mg/dL OR initiation of renal replacement therapy.

We tried to recognise *acute onset (AO)* (characterised by fever, fatigue, nocturnal sweats, weight loss and/or the triad of Löfgren syndrome: erythema nodosum, bihilar lymphadenopathy and arthritis) from *subacute onset (SO)* (cough, dyspnoea and/or chest pain as the most common symptoms) according to evidence from GenPhenReSa project (9). Patients with AO more often had bronchial and musculoskeletal involvement, but less frequent cardiac, hepatic or splenic involvement. Beyond these observations from GenPhenReSa project, we consider an progressive renal failure as a characteristic of SO. GenPhenReSa project classifies 5 phenotypes of sarcoidosis:

1) abdominal involvement (splenic/hepatic/renal);
2) ocular-cardiac-cutaneous (all but not erythema nodosum)-salivary glands-central nervous system-involvement;
3) musculoskeletal-cutaneous (erythema nodosum) involvement;
4) pulmonary and intrathoracic lymph node-involvement;
5) extrapulmonary involvement.

Renal involvement is placed in phenotype 1, but it could be present in all other phenotypes (11% in phen.1, 2% in phen. 2, 4% in phen. 3, 2% in phen. 4, 20% in phen. 5).

Statistical analysis

Analyses were performed using STATA version 13.1 (College Station, Texas, USA). Patients' characteristics were summarised in frequencies for categorical data or in means and standard deviations for continuous data. In case of missing values, frequencies were given for available data only. Fisher's exact test were used to compare characteristics between the groups. Chi^2 test was not adopted for little sample size. Univariate logistic regression models were used to examine the association between complete recovery and these variables: hypercalcaemia at RBx, fever as exordium symptom and lung symptoms at exordium. Significance was defined for a p-value < 0.05.

Results

Epidemiologic, clinical and histological characteristics of AKI-patients

We collected data of RBx of 39 patients (of which 3 Africans), 72% were men (28/39), 28% women (11/39). 36 patients presented with AKI (M/F=27/9, average age 59.2±12.6 years), 3 for nephrotic proteinuria (M/F=1/2, average age 56.0 years). Renal histological diagnosis was made in 9 patients already known to have a systemic sarcoidosis (23%, 9/39, a median of 86 months after the initial diagnosis, range 1week-516 months); among 30 new cases of disease (77%, 30/39) 26 had unacknowledged systemic sarcoidosis (87%), 4 had an isolated renal sarcoidosis (13%). Out of 18 patients with lung involvement, 7 patients were already aware of lung sarcoidosis diagnosis (39%), whereas in 11 patients lung involvement was documented in conjunction with RBx (61%).

In Table 2 and Figure 2 the most common symptoms at onset.

Among 36 patients with AKI, 86% (31/36) had sGIN-AKI (M/F=26/5; average age 59.4±11.7 years), 14% (5/36) had other patterns (No-sGIN) (M/F=1/4; average age 58.0±19.3 years, female average age 54.5±20.3 years, a male patient who was 72 years old): 3 patients presented tubular interstitial nephritis (TIN) without granulomas, one patient tubular microcalcifications and one patient chronic lesions. Considering 3 cases with nephrotic proteinuria, a 64 years old woman showed the association of sGIN and focal segmental glomerulosclerosis (FSGS) (uProt of 8.6 g/24h), the other 2 patients primary glomerular lesion with no interstitial involvement (a 65year old man with minimal changes disease and 39year old woman with fibrillary glo-

Table 2. Clinical features

Pt ID (n=39)	Symphoms at renal Bx	acute onset S	organs involved at renal onset
sGIN-AKI			
1	weakness, weight loss	0	kidney, lung
2	performance status decline, hepatosplenomegaly	1	kidney, liver, spleen
3	persistent cough, xerostomia, xerophthalmia	0	kidney, lung, eye
4	slight fever, dry cough	0	kidney, abdominal lymph nodes
5	uveitis	0	kidney, abdominal lymph nodes, eye
6	fever, persistent thoracoalgia, low back pain	0	kidney, lung
7	performance status decline, weight loss (10kg in 1yr), diarrhea, nausea, vomiting, ideomotor slowdown	1	kidney, liver, spleen, bone marrow
8	performance status decline, weight loss, dry cough,left iridocyclitis	0	kidney, lung, liver, spleen, abdominal lymph nodes, eye
9	transient fever, weakness, lack of appetite, weight loss	1	kidney, lung, abdominal lymph nodes
10	malaise	1	only kidney
11	dry cough, dyspnea, weakness	1	kidney, lung
12	performance status decline, fever	1	kidney, liver
13	strong weakness, generalised night itching, weight loss	0	kidney, mediastinum
14	slight fever, acute arthritis of knees and tibio-talus joints, erythema nodosum, hyperchromic urine	1	kidney, lung, joints, skin
15	weakness, weight loss, sicca syndrome, difficulty walking	0	kidney, lung
16	diarrhea, joint pain	1	kidney, lung , joints
17	performance status decline, dyspnea	0	kidney, lung
18	turbid urine	1	only kidney
19	weakness,performance status decline, erythema nodosum	1	skin, kidney
20	weakness, fever, low back pain	1	only kidney
21	hepatosplenomegaly,diarrhea, weakness, weight loss (5kg in 6months), left submandibular lymphadenopathy, persistent dry cough,	0	kidney, lung, cervical lymph nodes, liver, spleen
22	weakness, performance status decline, weight loss, nausea, dyspnea, heart-pounding, nocturia	0	kidney, pancreas, parotid
23		1	kidney, lymph nodes of mediastinum
24	weakness, fever, nausea, vomiting	1	kidney, lymph nodes of mediastinum, eye
25	dry cough	0	kidney, lung, lymph nodes of mediastinum
26	weakness, fever, nausea, drowsiness, myalgia	1	only kidney
27	weakness, performance status decline, nausea, weight loss, dyspnea, splenomegaly, anxiety	0	kidney, spleen
28	weakness, joint pain, fever, bilateral iridocyclitis, pollakiuria, papular erythema on hands and torso	1	kidney, joints, skin, eye
29	weakness, weight loss, performance status decline	0	kidney, lung
30		0	kidney, lung, spleen

Table 2. Clinical features

Pt ID (n=39)	Symphoms at renal Bx	acute onset S	organs involved at renal onset
31	myalgia	1	kidney, skin
No-sGIN-AKI			
32	itching,slight fever, weight loss	0	kidney, liver
33	sicca syndrome, weakness, weight loss, difficulty walking	1	kidney, lung, eye, lymph nodes of mediastinum, stomach
34	weakness,xerostomia,bilateral conjunctivitis, joint pain, slight fever, headache	1	kidney, eye, liver, spleen, abdominal lymph nodes
35	fever, lateral cervical and mediastinal lymphadenopathy, joint pain	1	kidney, lung, lymph nodes of mediastinum, peripheral lymph nodes, joints
36	abdominal pain spread to torso and right arm	1	kidney, lung, lymph nodes of mediastinum, abdominal lymph nodes
Nephrotic Syndrome			
37	weakness, erythema nodosum	0	kidney, skin
38		0	kidney, lung
39		0	kidney, lung

merulopathy, onset uProt 2.5 g/24h and 4.3 g/24h, respectively).
Basal comorbidities in Figure 3.

Laboratory findings at RBx of AKI-patients

AKI seemed to be more severe in sGIN patients than No-sGIN (RBx sCr 4.4±2.3mg/dl, uProt 0.7±0.5g/24h vs sCr 3.6±2.3mg/dl, uProt 0.6±0.3 g/24h, both p=ns; basal sCr: 1.1±0.4 vs 1.1±0.3, p=ns). 5 patients were affected by CKD before RS involvement (3 had sGIN-AKI and 2 No-sGIN-AKI, one patient for each group had reduced measure at renal ultra-sound).

According to KDIGO guideline (18), AKI severity was of stage 3 for 24 patients (21 in sGIN-AKI group and 3 in No-sGIN-AKI group), of stage 2 for 9 patients (8 in sGIN-AKI group and 1 in No-sGIN-AKI group), of stage 1 for 3 patients (2 in sGIN-AKI group and 1 in No-sGIN-AKI group) (p=ns) (Table 3, Figure 5). One sGIN-AKI patient had a necrotizing pattern and bone marrow involvement. This 50 years old man needed hemodialysis for anuria at onset. In Figure 4 there are altered urinary sediment of 27 patients (19 sGIN-AKI, 5 No-sGIN-AKI, 3 nephrotic proteinuria).

Treatment

The 36 AKI patients were treated with oral prednisone, with a dose ranged from 0.5 to 1 mg/Kg/day: 9 patients 0.5 mg/Kg/day, 5 patients 0.75-0.8 mg/Kg/day and 20 patients 1 mg/Kg/day; one patient received 8 mg/day of oral methylprednisolone, in one case steroid schedule wasn't available. Average steroid maintenance was similar between sGIN-AKI (15.7 months, range: 1-57) and No-sGIN-AKI patients (16.0 months, range 1–60). 10 out of 39 patients (8 sGIN-AKI, 2 No-sGIN-AKI) (26%) received intravenous steroid pulse therapy before oral steroids, but scheduled dose was not available. Considering AKI stage of 8 sGIN-AKI patients who received pulse therapy, 3 had stage 1, 2 had stage 2, 3 had stage 3; 2 No-sGIN-AKI who received pulse therapy had respectively stage 1 AKI and stage 3 AKI. For 34 patients (29 sGIN-AKI patients and 5 No-sGIN-AKI patients) RF was available after therapy (one patient was lost at FU, sCr was not available in another after steroid). After one month-steroid therapy sCr and

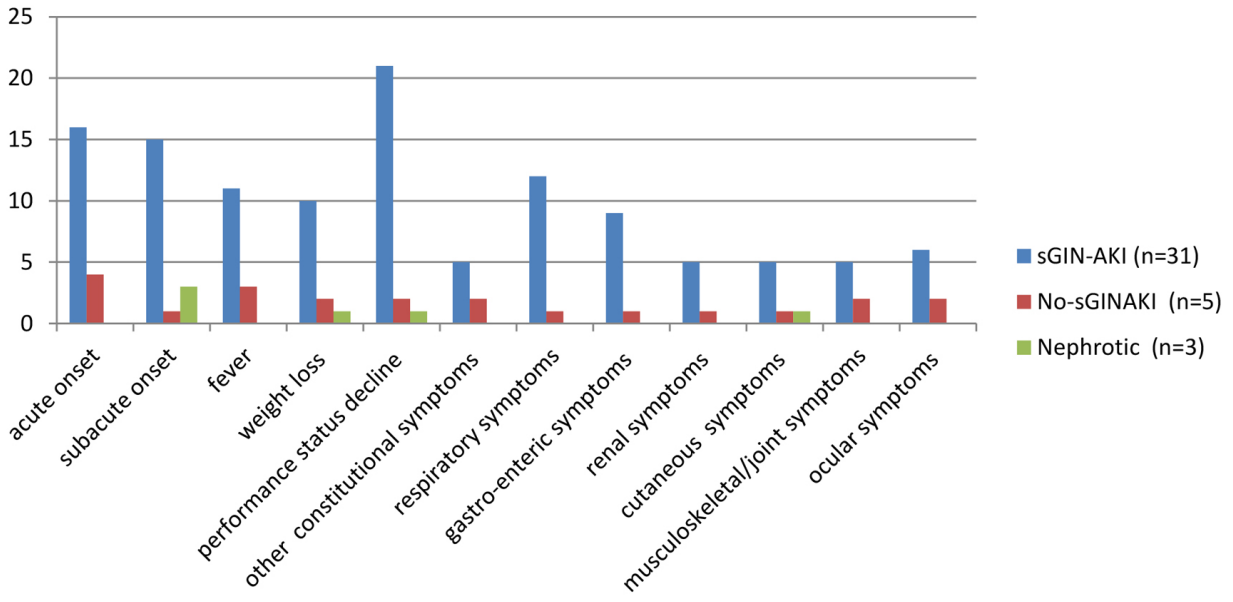

Figure 2 - Symptoms at exordium

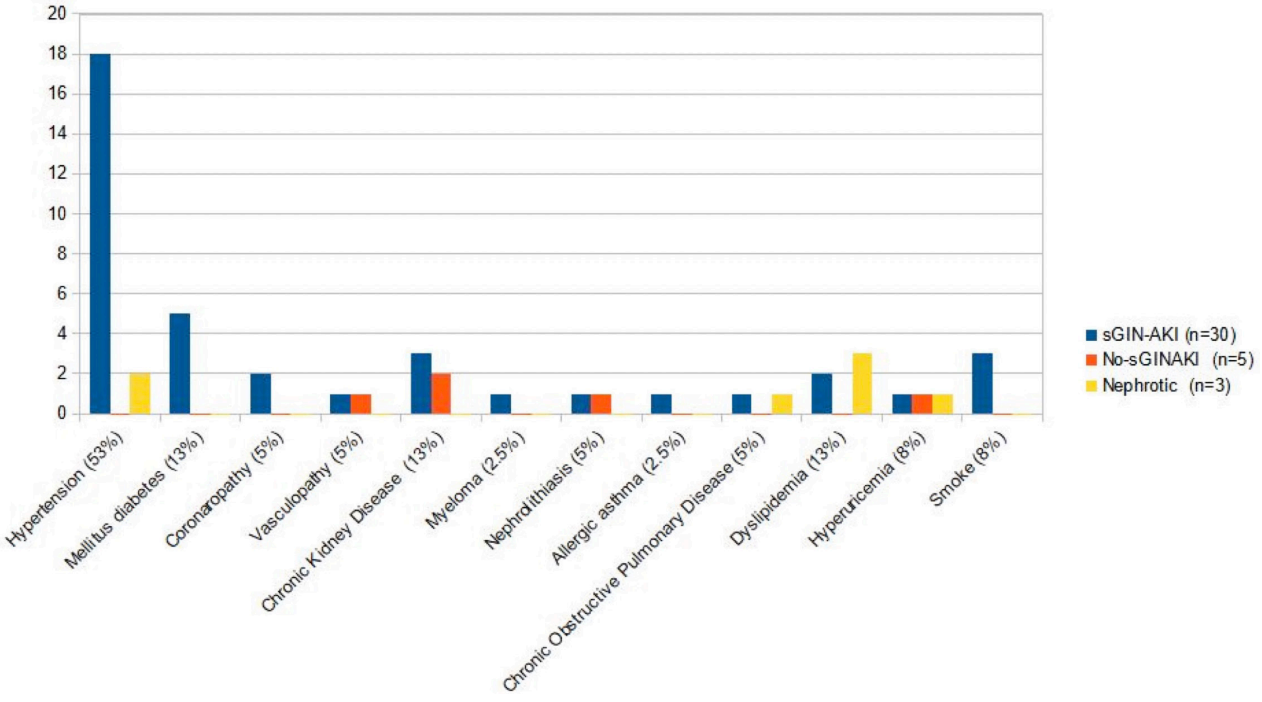

Figure 3 - A) Comorbidities. B) Basal comorbidities

uProt were both significantly lower in No-sGIN-AKI than in sGIN-AKI patients (sCr 1.2±0.2mg/dl and uProt 0.07±0.10 g/24h vs sCr 2.1±0.8mg/dl and uProt 0.38±0.29 g/24h, respectively p=0.009 and p=0.024). Nadir sCr during therapy was lower in No-sGIN-AKI than sGIN-AKI (respectively 1.1±0.1mg/dl vs 1.5±0.5mg/dl, p=0.057), as like as RF and uProt at last FU (sCr 1.1±0.1mg/dl and

Table 3. Renal function and outcome

Pt ID (n=39)	Renal onset	basal sCr	eGFR basal Cr	basal CKD	RBx age	RBx sCreat	StageAKI	Steroid ivBolus	Steroid Dose	1month sCreat	Steroid Min sCr (months)	Min sCr vsRBx	Partial Remiss	Complete Remiss	SteroidTp (months)	LastObs sCr	Last Obs eGFR	finalCKD	LastObs vsRBx (months)	Recurrences	postRBx AKI	exitus
sGIN-AKI																						
1	1	0,7	90	0	66	3	3	0	1	2,5	1,1	12	1	0	16	1,1	52	0	19	0	0	0
2	1	1,1	81	0	46	8	3	1	1	4,33	1,05	20	0	1	20	1,28	66	1	24	0	0	0
3	1	1,02	85	0	54	4,4	3	1	3	1,96	1,59	2	1	0	7	2	36		8	0	0	0
4	1	1,17	92	0	36	3,4	2	1	1	1,48	1,24	11	0	1	11	1,17	92	0	15	0	0	0
5	1	1,5	52	1	55	4,5	3	0	3	2,12	1,86	5	1	0	6	1,51	49	1	69	1	0	0
6	1	1,34	66	0	60	2,86	2	0	3	1,9	1,5	4	1	0	17	1,6	53	1	17	0	0	0
7	1	1,1	78	0	50	10,9	3	1	1		2,19	7	1	0	7	2,9	23	1	84			
8	1	1,1	75	0	56	2,45	2	0	1	1,86	1,3	29	1	0	57	1,57	47	1	69	1	1	0
9	1	0,9	73	0	53	2,1	2	0	3		0,9	6	0	1	12	0,9	68	0	122	0	0	0
10	1	1	73	0	76	5,2	3	0	2	1,5	1,5	1	1	0	12	1,6	39	1	110	0	0	1
11	1	1	77	0	68	3,4	3	1	3		1	3	0	1	3	1,8		1		2	0	0
12	1	1,2	67	0	55	5	3	0	3						24	3,9	14	1	300	1	0	0
13	1	1	74	0	75	2,83	2	0	3	1,58	1,28	3	1	0	3	1,28	54		3			
14	1	1,1	86	0	36	1,8	1	0	3	1,1	1.1	84	0	1	2	1,65	45	1	257	0	0	0
15	1	1,13	65	0	72	3,13	2	1	2	1,7	1,7	60	1	0	6	1,6	41	1	60	0	1	1
16	1	0,9	86	0	70	5,72	3	0	2	3,2	1,48	48	1	0	56	1,6	42	1	56	1	0	0
17	1	1,2	64	0	62	4,83	3	1	2	1,46	1,2	3	0	1	6	1,2	64		3			
18	1	0,91	101	0	48	3,05	3															
19	1	1,05	52	0	74	5,5	3	0	3	1,6	1	24	0	1	36	1,1	48	1	36	0	0	0
20	1	1,2	70	0	51	9,5	3	0	3	1,7	1,2	36	0	1	9	1,37	58	1	48	1	0	0
21	1	0,84	89	0	71	5,42	3	1	3		2		1	0	12	2	31	1	72	0	0	1
22	1	1,1	73	0	64	9	3	0	3	2,7	1,8	6	1	0	6	1,8	39		6	1	1	
23	1	1,1	70	0	65	2,3	2	0	1	1,3	1,1	6	0	1	6	1,1	71		6			
24	1	1,1	78	0	49	5,9	3	0	3	2,6	2,6	1	1	0	2	2,6	28		1			
25	1	1,1	77	0	51	3,5	3	0	3	4	2,78	6	1	0	6	2,78	25		6			
26	1	3,2	17	1	84	4,4	3	0	1	2,3	2	4	0	1	4	2	30		4			
27	1	1,2	60	0	72	3	2	0	3	1,7	1,2	24	0	1	33	1,2	59	0	24	0	0	0
28	1	0,5	126	0	54	2	3	0	1	1,71	1,1	24	1	0	48	1,2	67	0	36	0	0	0

Table 3. Renal function and outcome

Pt ID (n=39)	Renal onset	basal sCr	eGFR basal Cr	basal CKD	RBx age	RBx sCreat	StageAKI	Steroid ivBolus	Steroid Dose	1month sCreat	Steroid Min sCr	Min sCr vsRBx (months)	Partial Remiss	Complete Remiss	SteroidTp (months)	LastObs sCr	Last Obs eGFR	finalCKD	LastObs vsRBx (months)	Recurrences	postRBx AKI	exitus
29	1	1,2	66	0	59	3,6	3	0	3	1,83	1,2	64	0	1	2	1,2	64	0	121	1	0	0
30	1	0,9	69	0	61	4,92	3	0	3		2	3	1	0	3	1,9	28	1	25	0	0	0
31	1	0,8	88	0	47	1,5	1				0,8	2	0	1		0,9	74	0	52	1	1	0
No-sGIN-AKI																						
32	2	1,6	31	1	75	3,1	1	0	3	1,48	1,25	36	0	1	60	1,29	39	1	60	0	0	0
33	2	1,17	62	0	72	7,63	3	1	3	1,1	1,1	1	0	1	1	1,1	67		1			
34	2	0,77	95	0	43	2,64	3	1	1	0,93	0,93	1	0	1	1	0,93	95		1			
35	2	1,1	77	0	31	2,7	3	0	3	1,2	1,2	1	0	1	1	1,2	68		1			
36	2	1,0	58	1	68	2,06	2	0	2	1,04	1,04	1	0	1	17	1,15	48	1	48	1	0	0
Nephrotic Syndrome																						
37	3	0,64	113			0,6		0	2				0	1	9							
38	3	0,7	92		64	0,8		0	3				0	1	6	2,4	19	1	175	2	2	0
39	3	0,9	89		65	0,9		0	3				0	1	6	0,98		0	87	0	0	0

uProt 0.07±0.11 g/24h vs sCr 1.7±0.7mg/dl and uProt 0.49±1.04 g/24h, respectively (p=0.045 and 0.214) (Table 3 and 4, Figure 5). The 8 sGIN-AKI patients who received steroid pulse therapy before oral steroids didn't show statistical differences in sCr in confront with the 23 in oral steroids (sCr at RBx 5.1 vs 4.1 p=ns; one-month sCr 2.2±1.2 mg/dl vs 2.1±0.7mg/dl; p=0.46; at nadir 1.6±0.5mg/dl vs 1.7±0.7mg/dl; p=ns). No differences were observed in response between the 3 oral steroid schedules (0.5, 0.75-0.8 and 1 mg/Kg/day).

Primary endpoint: 24 patients had sCr available beyond the first year of FU (22 sGIN-AKI and 2 No-sGIN-AKI patients). Regarding of 22 sGIN-AKI subjects with final outcome (17 men and 5 women), 20/22 had basal normal RF and 2/22 had basal CKD. 13/20 (65%) starting with normal RF developed final CKD and 14/20 (70%) experienced a relevant RF worsening; only 7/20 (35%) had final normal RF (Table 5, Figure 6). Regarding 2 sGIN-AKI patients with basal CKD, they both maintained final CKD without worsening their RF. Considering 15 sGIN-AKI patients with final CKD at last FU, 73% (11/15) experienced AKI with most severe stage [final CKD, stage III vs stage I+stage II: 79% (11/14) vs 50% (4/8); p=ns]. Regarding of 2 No-sGIN-AKI subjects with final outcome, they both had basal CKD which maintained at last FU without worsening their RF.

Secondary endpoint: With steroid therapy all No-sGIN-AKI patients got CR (100% of cases, 5/5), in confront of 45% (13/29) of sGIN-AKI patients (p=0.047). Through an univariate logistic regression, lung symptoms at exordium resulted significantly related to CR: odds ratio 0.25, Std error 0.19, z -1.81, p 0.071 [IC 95% 0.06; 1.13], model p=0.063.

In Table 5 and Figure 6 endpoints are shown for sGIN-AKI patients with basal normal RF and FU >1 year.

Combined outcomes were considered only 21 of 22 sGIN-AKI patients with final outcome, because one patient (ID n.12) had sCr available beyond the 1° FU year but not data about initial

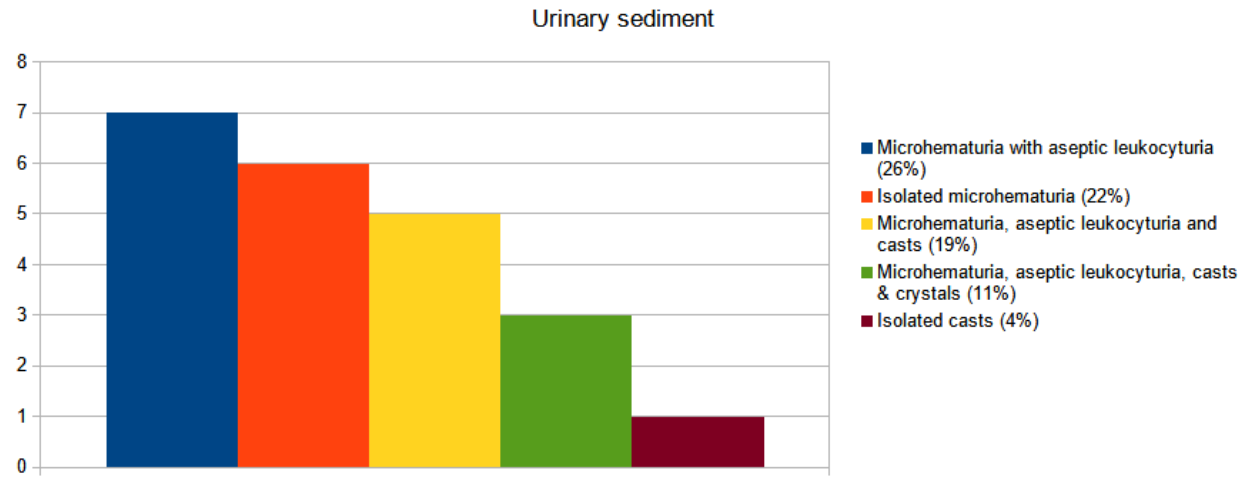

Figure 4 – Urinary sediment

response; among these 21 sGIN-AKI patients there were 2 patients with basal CKD and 6 patients with kidney relapse. Considering association of initial steroid response and final normal RF/final CKD: 29% (6/21) patients had CR and final normal RF (one with renal relapse), 48% (10/21) had PR and final CKD (3 patients with renal relapse, of whom one with basal CKD), 19% (4/21) had CR and final CKD (of whom one with basal CKD and 2 others with renal relapse), one with PR and final normal RF (4%, 1/21) (p=0.024). Considering association of initial steroid response and final RF worsening/not worsening: 33% (7/21) patients had CR and no final RF worsening (of whom one with basal CKD and

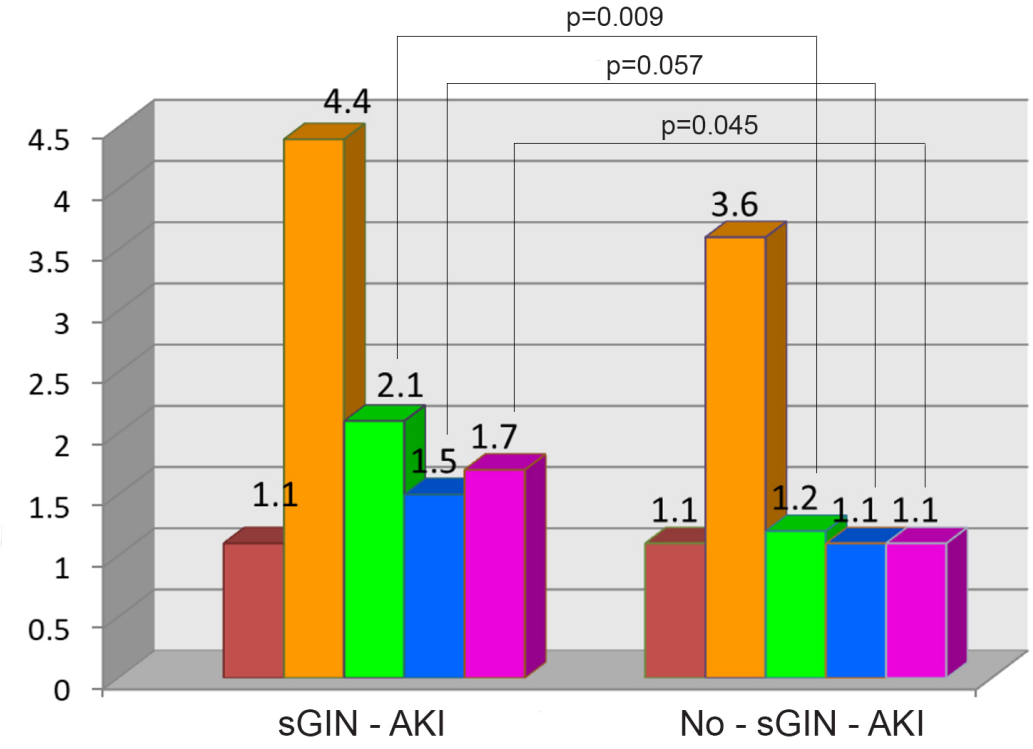

Figure 5 - Renal function

Table 4. sACE increase at RBx & proteinuria evolution

Pt ID (n=39)	SACE incr. at RBx	uProt RBx(g/die)	1month-uProt (g/24h)	LastObs uProt (g/24h)
sGIN-AKI				
1	0	0	0	0
2	0	0,23	0,15	0,1
3	1	1	0,58	0,4
4		0,5	0,14	0,23
5	0	0,46	0,27	0
6	0	0,3	0	0
7	1	0,35		3,9
8	1	0,46	0	0,2
9	1	0,3		0
10	0	1,1		0,4
11	0	1		
12	0	0,3		0,4
13		0,7		
14	0	1		4
15		0,328	0,49	0,31
16		0,84	0,7	0,16
17	0	0,7	0,4	0,01
18	0	0,3		
19	1	2	0,95	0,16
20	1	0,5	0,6	0
21		1,47		0,5
22	0	0,3		
23	0	1,3	0,28	0,15
24	0	0,6	0,7	0,7
25		1,5	0,6	
26	0	0,5	0,5	0,4
27	1	0,4		0
28	0	0,2	0,16	0
29		1,2		0,16
30	1	1,6		0,2
31		1,4		0,3
No-sGIN-AKI				
32	1	0,3	0	0
33	1	0,38	0	0
34	1	0,86	0,22	0,22
35	1	1		
36	1	0,7	0,06	0,04
Nephrotic Syndrome				
37	1	4,3	0,37	
38		8,6	7,5	0,34
39		2,48	0,5	0,34

another with renal relapse), 48% (10/21) patients had PR and final RF worsening (2 with renal relapse), 14% (3/21) with CR and final RF worsening (2 with renal relapse), 5% (1/21) patients had PR and no final RF worsening (he had basal CKD and even renal relapse) (p=0.008).

Women had lower sCr at RBx time (3.1±1.3mg/dl vs 4.7±2.4mg/dl, p= 0.028), after one month (1.5±0.6mg/dl vs 2.1±0.9mg/dl, p=0.061), at nadir (1.1±0.4mg/dl vs 1.5±0.5mg/dl, p=0.015) and at last observation (1.2±0.3mg/dl vs 1.7±0.7 mg/dl, p=0.010) than men, despite similar basal levels (1.0±0.3mg/dl vs 1.2±0.4mg/dl, p=0.092). This regarded also the sGIN-AKI subgroup at treatment nadir and at last observation (F vs M: sCr 1.2±0.5mg/dl vs 1.6±0.5mg/dl, sCr 1.2±0.4 mg/dl vs 1.8±0.7 mg/dl, respectively; p=0.055 and p=0.038). Even though statistical significance, it's interesting observing difference in eGFR between sex: at basal GFR is almost the same between sex (AKI patients, F vs M: basal 72.3±20.1 vs 73.7±19.0ml/min, p=0.429), during therapy women had always eGFR higher than men (at nadir 61.7±21.8 vs 54.6±18.9 ml/min, p=0.179; at last observation 57.8±20.4 vs 47.8±18.0 ml/min, p=0.087). The same observation considering only sGIN-AKI patients (F vs M: basal 74.4±15.5 vs 74.1±19.2ml/min, p=0.488; at nadir 58.6±23.3 vs 54.0±19.1 ml/min, p=0.321; at last observation 54.0±18.1 vs 47.0±18.0ml/min, p=0.217). Even if without significance, they had also better outcome than men considering all AKI patients [F vs M: CR 78% (7/9) vs 44% (11/25), p=0.125; final CKD 71 % (5/7) vs 69% (11/16), p=ns; RF worsening 29% (2/7) vs 69% (11/16), p=0.169] and sGIN-AKI subjects [F vs M: CR 60% (3/5) vs 42% (10/24) p=ns; final CKD 60% (3/5) vs 69% (11/16), p=ns; RF worsening 40% (2/5) vs 69% (11/16), p=ns].

AKI with hypercalcemia was milder than AKI without hypercalcemia at RBx (sCr 3.6±1.7mg/dl vs 4.5±2.2mg/dl, p=0.114), after one month (sCr 1.6±0.7mg/dl vs 2.2±0.7mg/dl, p=0.065), at nadir (sCr 1.2±0.2mg/dl vs 1.4±0.5mg/dl, p=0.038) and at last observation (sCr 1.3±0.3mg/dl vs 1.6±0.7mg/dl, p=0.071). 80% (8/10) patients with AKI with hypercalcemia got CR, while 45% (9/20) AKI patients without hypercalcaemia got CR (p=0.199); this was observed also considering sGIN-AKI patients: 66%

Table 5. EndPoints in sGIN-AKI

	sGIN-AKI pts basai NRF &> 1 year FU	sGIN-AKI pts basai NRF & > 1 year FU with:					
		AKl without hvoercalc.	Giant Cells	Acute onset	Subacute onset	Sevlnterstinfiltr	lnterstfibros
Total pts	20	13	11	11	9	8	8
Stage III AKI	60% (12/20)	62% (8/13)	82% (9/11)	73% (8/11)	44% (4/9)	63% (5/8)	75% (6/8)
Part al Recovery	50% (10/20)	62% (8/13)	45% (5/11)	36% (4/11)	66% (6/9)	63% (5/8)	63% (5/8)
Worsening RF	70% (14/20)	85% (11/13)	64% (7/11)	73% (8/11)	66% (6/9)	75% (6/8)	75% (6/8)
Final CKD	65% (13/20)	77% (10/13)	64% (7/11)	64% (7/11)	66% (6/9)	75% (6/8)	75% (6/8)

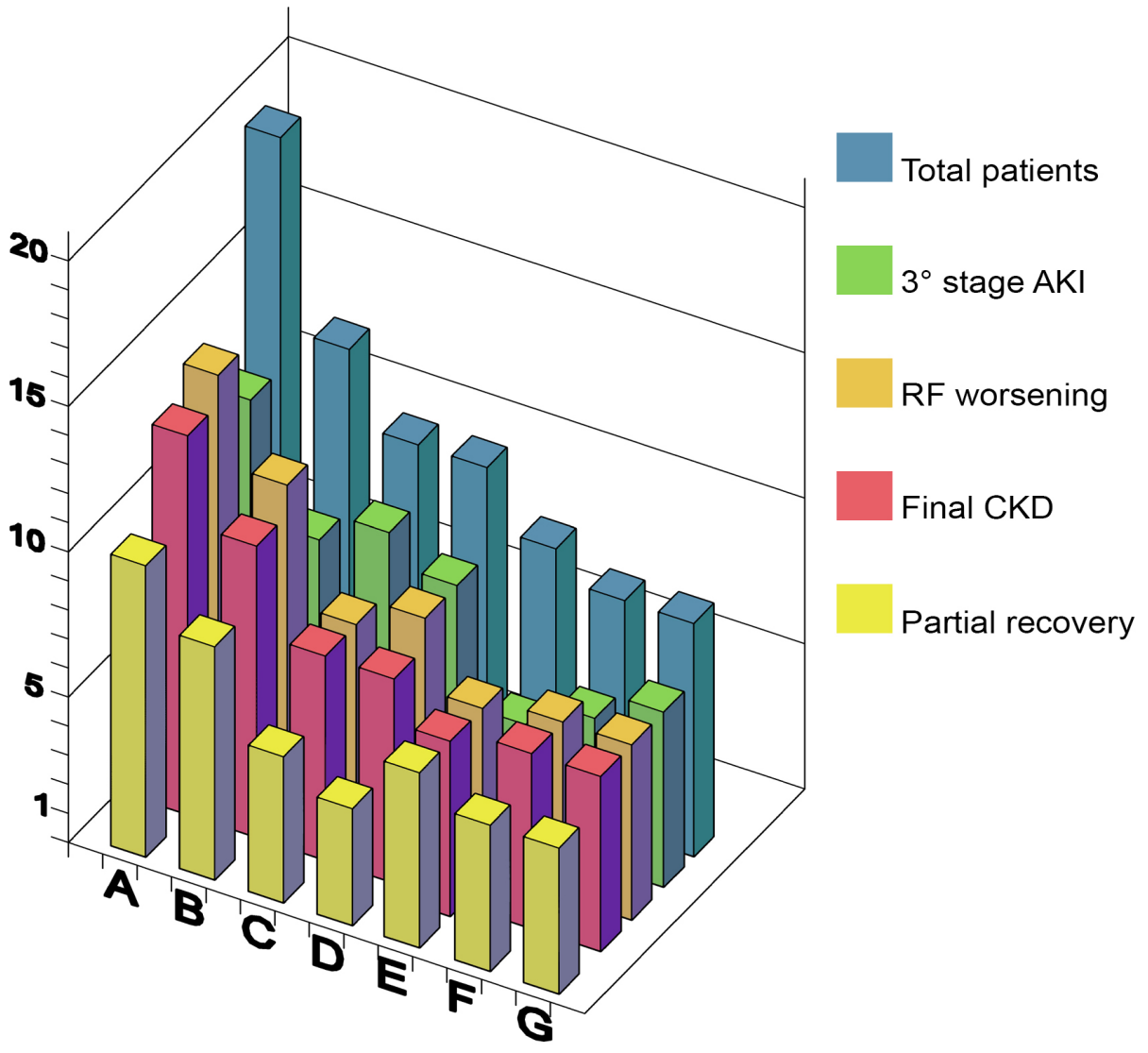

Figure 6 - EndPoints in sGIN-AKI. A:: all sGIN-AKI pts basal NRF & > 1 year FU; B-G: subgroups of sGIN-AKI pts basal NRF & > 1 year FU in the following subgroups: , B: AKI without hypercalcemia, C: Giant Cells, D: Acute onset, E: Subacute onset, F: Severe Interstitial Infiltrate; G: Interstitial fibrosis.

Table 6 - Calcium metabolism

Pt ID (tn=39)	Hypercalcemia (mg/dl)	Hypercalciuria (mg/die)	Calcemia (mg/dl)	Calciuria (mg/24h)	Phosforemia (mg/24h)	Phosphaturia (ng/ml)	Phosphaturia (ng/ml)	25OH D vitamin (pg/ml)	1-250H D vitamin (pg/ml)	PTH	Other Anomalies
sGIN-AKI											
1	0	0									
2	0	0	8,7								
3	0	0	9,33								
4	0	0	10,21	3,6						3	
5	0	0	9,02								
6	0	0	8,3								
7											
8	0	0	9,5	206	2,7	356				19	Tubular Microcalcifications
9	0	0									
10	0	0									
11	1	0	10	9							
12	0	0									
13	1	0	10,6								Intestitial microcrvstals deoosits
14											
15	0	0	8,9	235							
16	1	0	11	1	270						
17	0	0	9,7								
18	1	0	2	38 (mmol/L)							
19	0	0	9,4		4,6					31	
20	0	0	9,9		6					33	
21	0	0	10,1	67,5	4,9					38	
22	0	0	9,47	5,92						33	
23	1	0	12,2		3,5		19	19	220	4	
24	0	0	93		44					164	
25											
26	0	0	9,03		3,9		5	5	43	96	Urinarv crvstals: amorohous trio-leohosohate

Table 6 - Calcium metabolism

Pt ID (tn=39)	Hypercalcemia (mg/dl)	Hypercalciuria (mg/die)	Calcemia (mg/dl)	Calciuria (mg/24h)	Phosforemia (mg/24h)	Phosphaturia (ng/ml)	Phosphaturia (ng/ml)	25OH D vitamin (pg/ml)	1-250H D vitamin (pg/ml)	PTH	Other Anomalies
27	1	1	12,02	587	3,4	1221	13	13	175	3	Urinarv crvstals: calcium oxalate
28	0	0	8,83		4,17						Rare urinarv crvstals of urate
29	1	0	11,5	280	5,4					9	
30											
31	0	0	9		3						
NosGIN-AKI											
32	1	0	12,1	60	4,2					5	Tubular Micro-calcifications
33	1	0	13,83	10,8	71					21	
34	1	o	12,57								Tubular Microcalcifica-tions
35	o	o	88								
36	1	1	2,98 (mmol/L)								Tubular Microcalcific., lntestitial micro-crvstals deoosits
Nephro-tic Syn-drome											
37	1	o	2,29 (mmol/L)								
38	o	o	10,5		4,6					22	Rare urinarv crvstals, not soecified
39	o	o	98	201	43					22	

(4/6) of hypercalcaemic AKI patients got CR, against 42% (8/19) CR in AKI patients without hypercalcaemia (p=0.378) (Table 3, Table 6). Furthermore at univariate logistic regression, calcaemia at exordium resulted significantly related to CR: odds ratio 2.12, Std error 0.85, z 1.89, p 0.059, [IC 95% 0.97; 4.65], model p=0.027.

Considering sGIN-AKI patients with basal normal RF who had AKI without hypercalcaemia, 85% (11/13) had a worsening of RF at last FU >1 year from RBx and 77% (10/13) final CKD (Table 5, Figure 6).

sACE was available in 28 patients at RBx time (Table 4); its increase was associated with No-sGIN-AKI [100% (5/5) vs 35% (8/23) in sGIN-AKI, p=0.013], female sex [F vs M: 88% (7/8) vs 30% (6/20), p=0.011)], tubular microcalcifications [31% (4/13) with vs 0% (0/15) without, p=0.035]. After one month-steroid therapy sCr was significantly lower in AKI patients with high sACE levels at RBx than patients with normal sACE levels (1.5±0.4mg/dl vs 2.1±0.9mg/dl, p=0.018; at baseline 4.3±2.1mg/dl vs 4.7±2.9mg/dl, p=ns). CR was reached by 69% (9/13) patients with sACE increase and 46% (6/13) with no sACE increase at RBx (p=ns). No relationship was present between sACE and final outcome, neither in all AKI patients nor in sGIN-AKI subgroup.

Acute vs subacute onset: Even though acute onset (AO) was associated to CR than subacute onset (SO) [CR, AO vs SO: 72% (13/18) vs 31% (5/16), p=0.037; CR in sGIN-AKI patients, AO vs SO: 64% (9/14) vs 27% (4/15), p=0.066; 2 patients lost at FU], no differences in final outcomes in 21 patients having sCr available beyond the 1° FU year, even in sGIN-AKI patients with basal normal RF (Table 5, Figure 6). Steroid response for SO was a predictive factor final outcome: 30% (3/10) patients with CR got final normal RF, 70% (7/10) patients without CR got final CKD (of whom one with basal CKD) (p=0.008), no patient with CR and final CKD or with PR and final normal RF.

Fever at exordium: In this context fever was associated with higher probability of CR, considering either all AKI patients (29sGIN-AKI+5No-sGIN-AKI) [CR, fever vs no fever: 83% (10/12) vs 36% (8/22) p=0.013] or patients with presence of interstitial infiltrate [CR, fever vs no fever: 89% (8/9) vs 25% (3/12), p=0.008; also at high degree: CR, fever vs no fever: 86% (6/7) vs 0% (0/6), p=0.005], age > 50 years [fever vs no fever: 83% (5/6) vs 33% (7/21), p=0.060] or sGIN-AKI patients [CR, fever vs no fever: 78% (7/9) vs 30% (6/20), p=0.041] and these sGIN-AKI subgroups: interstitial infiltrate at RBx [CR, fever vs no fever: 83% (5/6) vs 25% (3/12), p=0.043], high degree interstitial infiltrate [CR, fever vs no fever: 80% (4/5) vs 0% (0/6), p=0.015], age > 50 years at RBx [fever vs no fever: 80% (4/5) vs 26% (5/19), p=0.047].

Through an univariate logistic regression, fever as exordium symptom was related to CR: odds ratio 6.5, Std error 5.81, z 2.09, p 0.036, [IC 95% 1.13; 37.48], model p > chi^2 0.021.

Fever at exordium was not associated with a particular final outcome.

GenPhenReSa phenotypes & outcome in Table 7.

Histological parameters:

A complete detailed histological description was available only in 25 patients: 58% (18/31) sGIN-AKI, 100% (5/5) No-sGIN-AKI, 66% (2/3) nephrotic pa-

Table 7 - GenPhenReSa phenotypes outcomes

Partial Recovery	**1 phenotype**	**2 phen.**	**3 phen.**	**4 phen.**	**5 phen.**
sGIN-AKI after steroid therapy (n=29)	67% (4/6)	86% (6/7)	33% (1/3)	43% (3/7)	33% (2/6)
No-sGIN-AKI after steroid therapy (n=5)	100%(2/2)	100%(1/1)	100%(1/1)	100%(1/1)	100%(1/1)
sGIN-AKI pts basai NRF & > 1 year FU (n=17)	**1 phenotype**	**2 phen.**	**3 phen.**	**4 phen.**	**5 phen.**
Total pts	7	3	2	5	3
Stage lii AKI	5	1	1	3	2
Part ili Recovery	4	2	1	2	1
Worsening RF	5	2	2	3	2
Final CKD	5	1	2	3	2

tients; on average glomeruli were 15 (range 3-38), only 3 biopsies had <6 glomeruli. For other patients we received the concise diagnosis of pathological reports. Immunofluorescence was mostly negative: one No-sGIN patient presented IgM not specific deposits, 3 sGIN patients presented glomerular C3 deposits (mesangial and pericapsular) and No-sGIN minimal change patient presented arteriolar C3 deposits.

We tested giant cells, interstitial fibrosis and severe interstitial infiltrate as histological predictors of steroid response in terms of RF in AKI patients (Table 8).

Evaluation of **interstitial infiltrate** grade was available in 17 sGIN-AKI patients and in 5No-sGIN patients. Severe interstitial infiltrate (SII) showed a trend with association to worse prognosis, both in terms of recovery [CR, no SII vs SII: 78% (7/9) vs 46% (6/13) p=ns] and primary outcome [final CKD, no SII vs SII: 66% (4/6) vs 80% (8/10), p=ns; final RF worsening no SII vs SII: 33% (2/6) vs 60% (6/10), p=ns]. Considering 8 sGIN-AKI patients with basal normal RF, severe interstitial infiltrate and FU>1year, had PR in 63 % (5/8) and final CKD in 75% (6/8) (Table 5, Figure 6).

Interstitial fibrosis (IF) and final CKD showed a trend in association: 60% (3/5) of patients without interstitial fibrosis got final CKD, whereas 83% (10/12) of patients with interstitial fibrosis got final CKD (p=ns). Considering 8 sGIN-AKI patients with basal normal RF, interstitial fibrosis and FU>1year, had PR in 63% (5/8) and final CKD in 75% (6/8) (Table 5, Figure 6).

Giant cells (GC) were presented in 48% (15/31) sGIN-AKI patients [83% (15/18) of sGIN-AKI patients with complete pathological report] and were almost presents in all GenPhenReSa phenotypes: 75% (3/4) phenotypes 1, 2 and 4, 100% (3/3) phenotypes 3, 66% (2/3) phenotypes 5.

GC were important prognostic histological parameters, as severe interstitial infiltrate and interstitial fibrosis. Considering 11 sGIN-AKI patients with basal normal RF, GC and FU>1year, had PR in 45% (5/11) and final CKD in 64% (7/11) (Table 5, Figure 6).

GC sGIN-AKI patients and combined outcome: There were 13 GC sGIN-AKI patients with

Table 8. Histology

Pt(n=39)	Glomeruli numbers	% Glomerular Sclerosis	Tubular Atrophy Grade	Intratubular Casts	Tubular Infiltrate	Epithelioid cells	Giant Cells	Interstitial Infiltrate Grade	Necrotic Granulomas	Interstitial Fibrosis	Interstitial Fibrosis Grade	Arteriolar Sclerosis	Arteriol Lumen Reduction	Arteriolar Fibrinoid Necrosis
sGIN-AKI														
1	7	0,14	1		0	0	2	1	0	0		0		
2	7	0	0	0	0	1	1	3	0	1	1	0	0	0
3	17	0	3		1	1	0	3		1	1	0	0	0
4	4	0,25	1	0	0	1	1	3	0	0	0	0	0	0
5	13	0,31	3	1	1	1	1		0	1	1	1	0	0
6	23	0,04	3	0	1	1	1	3	0	1	2	1	0	0
7	12	0	0	0	0	1	1	3	0	1	1	0	0	0
8	0													
9														
10														
11														

Table 8. Histology

Pt(n=39)	Glomeruli numbers	% Glomerular Sclerosis	Tubular Atrophy Grade	Intratubular Casts	Tubular Infiltrate	Epithelioid cells	Giant Cells	Interstitial Infiltrate Grade	Necrotic Granulomas	Interstitial Fibrosis	Interstitial Fibrosis Grade	Arteriolar Sclerosis	Arteriol Lumen Reduction	Arteriolar Fibrinoid Necrosis
12														
13	13	0	1		0	1	0	3	0	0	0	1	0	0
14	3	0,33	0	0	1	1	1	2	0	1	2	0	0	0
15	20	0	1	1	1		1	3	0	0	0	1	0	0
16			1			1	1	3	0	1	1	1	0	
17	13	0,07	2			1	1	1		0	0	1	1	0
18	27	0,15	1	1	1	1	1	3	0	1	1	0	1	0
19	16	0	3		1	1	1	3	0	1	3	1	1	0
20	10	0	3		0	1	1	3	0	0	0	0	0	0
21	38	0,13	2		0	1	0	3	0	1	2	1		0
22														
23														
24														
25														
26														
27														
28														1
29	10	0,3	3		0	1	1			1	1	1	0	
30	1	1	1											
31	12	0	1	0	1	1	1	1	0	0	0	0	0	0
No-sGIN-AKI														
32	3	0,33			0	0	0	3	0	1	3			
33	25	0,33	1	0	0	0	0	0	0	1	2	1	1	0
34	20	0	1	1	1		0	1		0	0	0	0	0
35	6		1	0	0	0	0	3	0					
36	17	0,35				0	0	1	0	1	1	0	0	0
Nephrotic Syndrome														
37														
38	25	0,08		1		1	0	1	0	0	0	0	0	0
39	17	0		1	1		0	1				0	0	0

FU >1 year, of whom 2 with basal CKD and other 3 patients with renal recurrence. 31% (4/13) patients had CR and final normal RF, 46% (6/13) patients had PR and final CKD (one with basal CKD, 2 patients with renal relapse), 23% (3/13) patients had CR and final CKD (one with basal CKD, one with renal relapse), nobody with PR and final normal RF (p=0.070).

All 9 GC sGIN-AKI patients with final CKD had interstitial infiltrate and 78% (7/9) interstitial fibrosis (of whom the 2 patients with basal CKD).

sACE increase in GC sGIN-AKI patients: patients having GC sGIN-AKI and sACE increase at RBx presented more severe AKI than patients without No-sACE increase (sCr 7.7±2.9mg/dl vs 4.2±2.2mg/dl, p=0.030). In 8 GC sGIN-AKI patients with sACE available, sACE increase wasn't related to outcomes (data not shown).

Serious adverse event, end stage renal disease and mortality, relapses

We had no data about steroid adverse side effects. Nobody of our series had to undergo chronic haemodialysis treatment. 3 sGIN-AKI patients died during FU: one for lung cancer at the age of 85 years (pt ID n.10), 9 years after RBx; one for pneumonia at the age of 77 years, 6 years after RBx (pt ID n.21), one for unknown cause at the age of 82 years, 10 years after RBx (pt ID n.15). 28% (11/39) patients had relapses: 9 sGIN-AKI patients, one No-sGIN-AKI patient and a patient with sGIN and nephrotic syndrome at exordium. 13% of sGIN-AKI patients (4/31) had another AKI post-RBx. For 2 patients AKI occurred after steroid stop: for case n.15 AKI occurred after 2 years from steroid stop (sCr from 1.7 to 2.1mg/dl), with a subsequent spontaneous RF increase; for case n.22, stage III AKI (sCr up to 4.5mg/dl, uProt 0.2 g/24h) occurred after 3 months from RBx and a premature steroid stop. A new steroid restart plus azathioprine got PR (at 6moths from RBx sCr 1.8mg/dl). For other 2 patients AKI occurred on steroid maintenance: for case n.8 during 3° therapy year: in 4 months sCr crept up from 1.3 to 1.85 mg/dl, eGFR from 98 to 58 ml/min, calcaemia from 9.7 to 10,5 mg/dl, sACE from <40 to 125 U/l. After prednisone increase from 5mg alternating days to 25 mg every day, in 3 months sCr settled down at 1.53mg/dl, eGFR to 60, calcaemia to 10.2, sACE to 54. For case n.31 during 1° year (8mg metilprednisolone daily), after 10 months from RBx, hypercalcemic AKI occurred (sCr up to 2.7mg/dl, calcaemia12.1mg/dl). Thanks to 75 mg prednisone a day she got CR (sCr 0.96 mg/dl in 2 months).

Among 9 sGIN-AKI patients with relapse there was a patient with isolated renal sarcoidosis: he had a renal recurrence 2 years later RBx. No-one of isolated sGIN-AKI patients developed a systemic disease during a average FU of 54 months (4-110 months).

Response in nephrotic patients: One case of necrotizing sGIN and FSGS, presenting nephrotic syndrome, was treated with 1 mg/Kg/day oral prednisone, reaching CR after 24 months (uProt 65 mg/24h). The patient with minimal changes nephropathy and the one with fibrillary-pattern were respectively treated with 1 and 0.8 mg/Kg/day oral prednisone, reaching respectively one month-uProt of 500 and 370 mg/24h. Patient with minimal changes disease reached a 120 mg/24h-uProt after 24 months-therapy.

Discussion

Sarcoidosis diagnosis relies on compatible clinical manifestations, histological and radiological findings. GIN is not a pathognomonic lesion of sarcoidosis, so to conclude a case as sarcoid-GIN means to have considered all alternative diagnosis, excluding all causes of granulomatous inflammation. In our study, every patient was found compatible for a renal involvement of sarcoidosis thanks to a comparative analysis between clinical history and pathological findings, besides the absence of a significant immunofluorescence at RBx. In order to be more confident about diagnosis we considered elements listened in Table 1.

In literature there are important studies on renal sarcoidosis including not only sGIN but also TIN without granuloma and renal calcinosis lesions, as the epidemiological Japanese study (16 patients out of 14,191 RBx cases), where TIN without granulomas were observed in 19% (3/16), and renal calcinosis in 13% (2/16) (19).

We didn't use *sACE increase* to make the diagnosis more likely, since sACE is more useful for discarding sarcoidosis than for confirming it: from

the US population-based cohort of Olmsted County, Minnesota (295 patients with 251 ACE tests) increase sACE for diagnosis got a 41.4% sensitivity, a 89.9% specificity, a 25.4% positive predictive value and a 94.9% negative predictive value (9.2% prevalence among the tested) (20). A higher sACE sensitivity, range from 60% (21) to 78% (22), is less reliable because derived from study conduced in tertiary care centers. Moreover there are more sensitive and specific markers to confirm sarcoidosis (sIL-2R, CRP, SAA and chitotriosidase) (23). In our study *renal biopsy* was the key instrument in diagnostic process for many patients. In fact, RBx permitted diagnosis of unknown sarcoidosis in 74% (29/39), where in 23% (9/39) the kidney was the only organ in which a systemic sarcoidosis disclosed, thus suggesting that sarcoidosis is often a hidden disease, first manifesting itself at renal level, with an unexplained sCr rising, also without significant uProt, urinary sediment alterations (31% of our patients had an indifferent urinary sediment also in presence of AKI) or systemic symptoms. RBx proves how AKI encompasses both impairment (loss of function) and injury (structural damage) (24). Unexpectedly, 44% (8/18) patients with lung sarcoidosis at diagnosis were without respiratory symptoms, that is 21% (8/39) of all our patients. Not always a lung involvement give rise to dry cough, a frequent symptom in sarcoidosis. Our data strengthen previous observations: sarcoidosis diagnosis was possible thanks to RBx in 47% (8/17) in Rajakariar's monocentric study in London (25) (17 patients, 13 sGIN and 4 TIN without granulomas) and 49% (23/47) in Mahevas's multicentric French study (15) (47 patients, 10 TIN without granulomas and 37 sGIN, of whom 59%, 22/37, with giant cells): these patients had an unexplained renal impairment and were subsequently diagnosed with sarcoid renal involvement and systemic disease.

Our data confirm Mahevas's observation that renal sarcoidosis mostly occurs at the presentation of disease (in our series 77%, 30/39, in Mahevas's 81%, 38/47): in our series only 4 patients (10%, 4/39) had renal sarcoidosis during a systemic disease lasting more than 1 year, 5 patients (13%, 5/39) had exordium symptoms less than 12 months from RBx. We observed 4 patients with isolated sGIN: 13% (4/31) of sGIN-AKI patients and 10% (4/39) of all patients, in line with Löffler's series, with isolated sGIN frequency of 15% (4/27) (14). In Berliner's series, the widest sGIN-AKI retrospective study, isolated sGIN had a higher frequency: 30% (28/94) (26).

Regarding sGIN-AKI onset, RF at presentation reported in our survey was similar to the one reported by Berliner: average sCr and 24h-uProt at presentation were respectively 4.4 and 0.7 g/24h in our survey and 4.8 mg/dl and 1 g/24h in Berliner's study (26).

As previous studies, our study there isn't a correlation between uProt level and histological findings (27); nevertheless, a 24h-uProt >2.0 g/24h is associated with a glomerular involvement of sarcoidosis, also in course of sGIN. Interestingly, we included a patient with 8.6 g/24h due to necrotizing sGIN and FSGS, a histological finding not frequently reported in literature. For example, Berliner described two sGIN cases respectively characterized by mesangioproliferative glomerulonephritis and membranous nephropathy; the first presenting 4.3 g/24h-uProt and the second 6.9 g/24h at exordium (26).

The important findings of our survey are that some renal histological patterns (No-sGIN-AKI), hypercalcaemia at presentation and female sex are predictors of clinical recovery, whereas initial steroid response in GIN-AKI patients is predictive of final outcome. Though we can't exclude that *No-sGIN-AKI* patients might present sparse sarcoid granulomas in other areas of kidneys, missed in a kidney biopsy specimen (sampling bias), No-sGIN-AKI patients had a better renal outcome than sGIN-AKI subjects regarding AKI-stage, RF after 1 month therapy and steroid response: all No-sGIN-AKI patients had CR, reached by only 45% (13/29) sGIN-AKI patients. That suggested us to consider our 5 No-sGIN-AKI patients as cases of renal sarcoidosis different from granulomatous ones: 4 cases presented TIN without granulomas, 3 of whom had hypercalcaemia and tubular microcalcifications (n.32, 34 and 36) and one patient (n.36) with even hypercalciuria.

We could assume the mechanisms of renal impairment in 3 hypercalcaemic No-sGIN-TIN-AKI patients was inflammatory response to calcium deposits (observed even in interstitium in pt n.36). Patient n. 35 had TIN without calcium anomalies but a great inflammatory infiltrate (lymphocytes, plasma cells, neutrophils and eosinophils) with tubulitis and signs of tubular injury (intratubular microabscess and loss of hair brush edge). This patient could have a toxic proximal tubule damage, as the one described

by Sanada in his study (43 kidney biopsy of different causes of TIN). He observed with immunohistochemistry lysozyme in proximal tubular cells of 6 patients with renal sarcoidosis, 1 with chronic myelomonocytic leukemia (CMML) and 3 with idiopathic TIN (28). All sarcoidosis patients had a slightly increase in seric lysozyme. Sanada suggested that excessive lysozyme produced by monocytes/macrophages, filtered and reabsorbed by proximal tubular cells, gives rise to sufferance of proximal tubular cells for increased lysosomes activity to degrade lysozyme, hence the severe TIN, similar to the kidney injury in CMML (29).

Only one patient with No-sGIN-AKI (pt ID n.33) presented chronic lesions, not consistent with renal sarcoidosis: nevertheless, we included him because he had already known systemic sarcoidosis before RBx (not caseous granuloma in mediastinum), the disease stroke typical organs (lungs and eyes), AKI occurred in course of hypercalcaemia. Generally *hypercalcaemia* occurs in 11% of patients with systemic sarcoidosis (30) and in 20% in case of sGIN-AKI, as reported by Berliner (26). We observed a higher frequency in our series (28%, 11/39), in line with the one observed by Mahevas (34%) (15). In our patients there was the highest frequency of hypercalcaemia in No-sGIN-AKI patients (80%, 4/5) than in sGIN-AKI patients (23%, 7/31) and nephrotic patients (33%, 1/3). Probably 25(OH)D3–1αhydroxylase activity of sarcoid macrophages, generally observed in immunohistochemistry in granulomas (31), in our No-sGIN-AKI patients is extra-renal. In our survey *AKI with hypercalcemia* was milder than AKI without hypercalcemia, for an immediate recoverable functional kidney damage. Our data are in line with Mahévas's ones (15): in his study hypercalcemia was strongly correlated with CR at 1 year ([OR] 16; 95% [CI], 1.8-137, p= 0.003) and in a multivariate analysis hypercalcaemia was independently correlated with CR (OR = 18.9, p= 0.001). On the other hand, we observed that patients with *tubular microcalcifications,* despite a good immediate recovery, had final CKD (75%, 3/4), independently from the presence of sGIN-AKI or No-sGIN-AKI. That's why calcium precipitates cause a structural damage superimposed to the functional damage that is more refractory to steroid therapy. Moreover, hypercalcaemic AKI was not a guarantee for not relapse in our series (57%, 4/7, of hypercalcaemic sGIN-AKI got relapse), where in Mahévas's series no relapse was observed in patients with hypercalcaemia (15). Patients with sarcoidosis have normal concentration of seric 25D and increased 1-25D, since sarcoid-associated macrophages metabolizes 25D to 1,25D thanks to 25(OH)D3–1αhydroxylase (32). Baughman reported that the serum level of 1-25D was higher with sarcoidosis associated hypercalcaemia (33). In our series we could confirm these data, unfortunately in very few patients: among only 3 sGIN-AKI patients with both 25D and 1-25D seric levels, we had 2 patients with deficient 25D, increased 1-25D and hypercalcaemia (Table 6). We had only 2 patients with hypercalciuria, 5% (2/39), a very low frequency considering other studies, which affects 20-40% of patients: for instance, Mahevas observed hypercalciuria in 32% (15/47), complicated by nephrolithiasis in 3 cases and nephrocalcinosis in 1 case (15).

In our study 84% (M/F=26/5) of patients with sGIN-AKI patients were men, a proportion significantly higher that 64% (M/F 60/34) reported in Berliner's study (26). In our survey *women* seemed to have better response than men: this fact can't be explained only with the majority of No-sGIN cases affected female, because better outcome is evident also only considering sGIN-AKI subgroup. We aren't able to explain this finding, requiring further investigations. Surely, a hormonal different milieu is a important factor for immune system, hence a different susceptibility to disease.

Of note we had a lower prevalence of *CKD* before renal sarcoidosis involvement (5/39, 13%) than observed in other studies. This points out an acute sarcoid damage in our patients independent from other coexisting causes of renal injury. In fact, regarding to our patients with a FU longer than 1 year and basal normal RF, we observed final CKD in 63% (12/19). A very high frequency of *final CKD* is in line with Berliner's (26) and Mahévas's study, where at the end of FU there were 67% (31/46) patients with eGFR <60ml/min [n.20 CKD stage III, n.8 stage IV, n.3 stage V, of whom 2 patients necessitated hemodialysis (15)]. Even in Kamata's Japanese epidemiological study 80% (12/15) cases exhibited residual renal dysfunction and 53% (8/15) had CKD (Stage 3b/4) (19). Overall, the renal outcomes of renal sarcoidosis is not reassuring, generally with *final CKD is >60%,* even considering studies with population ethnicity different: in Kamata's 100% patients were Japanese

(19), in Mahévas's 68% (32/47) were of African or Caribbean origin and 32% (15/47) Caucasian (15); in our series 92% (36/39) were Caucasian.

It could be debated whether eGFR decline in CKD is caused by renal sarcoidosis or by *basal systemic pathologies.* In our series we focused our attention on these 7 factors in 20 sGIN-AKI patients with basal normal RF: 5 basal comorbidities (arterial hypertension, mellitus diabetes, coronaropathy, vasculopathy, myeloma) and 3 cardiovascular risk factors (smoking, dyslipidemia, hyperuricemia). 8 patients had no basal factors (score 0), 12 patients had one or more basal factors (score ≥ 1). Overall, 50% (10/20) patients with score ≥ 1 and final CKD, 10% (2/20) score ≥ 1 and final normal RF, 15% (3/20) score 0 and final CKD, 25% (5/20) score 0 and final normal RF (p=0.062). Considering hypertension, the more frequent comorbidity, in 19 sGIN-AKI patients with basal normal RF: 47% (9/19) patients with hypertension and final CKD, 11% (2/19) hypertension and final normal RF, 16% (3/19) without hypertension and final CKD, 26%(5/19) without hypertension and final normal RF (p=0.074). Trend in association between comorbidities and final CKD disappeared grouping patients in score 0-1 (basal comorbidities/ risk factors 0 or 1) and score ≥2 (basal comorbidities/risk factors ≥2, data not shown). This study was not designed to stratify patients by uProt and further adjusting for various time-varying confounders, so the role of comorbidities in CKD progression can't be surely defined. Generally, the annual age-related decline in eGFR for people older than 40 years is 1 mL/min/1.73 m^2 in the background population without CKD (34, 35, 36) down to 0.4 mL/min/1.73 m^2 in a healthy population of white ethnicity and without diabetes (37). 5 ml/min/year is the threshold defined as rapid progression as rate by KDIGO guidelines (17). In our sGIN-AKI patients with >1year-FU, average annual eGFR decline is 4.9ml/min (average FU 6.6 years), 5.6 ml/min only sGIN-AKI patients with normal basal RF, basal hypertension and not diabetic, 6.8 ml/min in patients of this last group progressing to CKD. Fast decliners who developed incident CKD are patients generally with type 2 diabetes, hypertension, older age, African-American race (38) or affected by type 1 diabetes, as per Joslin Clinic cohort (39). Our patients don't have a similar burden of comorbidities which justify a fast eGFR decline, so we suppose that sarcoidosis damage is more important than basal comorbidities in reaching CKD.

Regarding to final outcome our data can't confirm our expectation of higher frequency of final CKD in sGIN-AKI patients than in other sarcoid kidney patterns, due to little sample of No-SGIN-AKI patients. In literature, in 27 German patients with renal sarcoidosis, Löffler observed that 30% patients with sGIN was more often associated with advanced stages of CKD than any other histological patterns (14). Conversely, in Rajakariar's (25) and Mahévas's studies (15) TIN without granulomas had a similar response to steroid therapy than sGIN. Nevertheless, we observed an important peculiarity of sGIN-AKI, where initial steroid response was predictive of final outcome: a third of sGIN-AKI patients had good combined outcomes [29% (6/21) CR and final normal RF, 33% (7/21) CR and no final RF worsening], half of patients bad combined outcomes [48% (10/21) PR and final CKD, 48% (10/21) PR and final RF worsening]. The group of sGIN-AKI patients with initial CR and final CKD (19%, 4/21) could appear as a paradox, but 2 patients had disease recurrence after steroid therapy and a patient had a basal CKD. Only a man developed CKD during aFU lasting 257 months (pt ID n.14). The peculiar distribution of sGIN-AKI patients according to combined outcome was significantly maintained in subgroup of sGIN-AKI patients with giant cells: a third of GC sGIN-AKI patients had good combined outcomes, half of patients bad combined outcomes (details not shown). Our observation are in line with Mahévas study, where a complete response to steroid at 1 month is strongly correlated both with response at 1 year ([OR] 7; 95% [CI], 1.6-44.8, p< 0.001) and with a CR at the end of FU ([OR] 7.6; 95% [CI], 2-41, p< 0.001) (15). In Mahevas's series patients with an unfavourable response at 1 month were more susceptible to relapse at the end of FU, observation not confirmed in our series (data not shown).

In our survey *giant cells* were important prognostic histological parameters, as *severe interstitial infiltrate* and *interstitial fibrosis*. In Berliner'study repeated kidney biopsies in patients with bad renal outcome after steroid therapy showed increased interstitial fibrosis as CKD sign, whereas in Mahévas's study interstitial fibrosis is the only pathological finding that correlates with renal outcome. In our opinion the worse prognosis of sGIN could be due to the

high pro-inflammatory and pro-fibrotic pathways activated by epithelioid cells in sarcoid granulomas (40, 41) and GC located in them (42). So it's comprehensible why our sGIN-AKI patients with GC presented 3° stage AKI in 66% (10/15), interstitial infiltrate in 100% (15/15) and interstitial fibrosis in 75% (9/12). The key challenge if steroid manages to stop intense T-helper 1 response, whose GC are both the result and a contributing cause (43), and consequent proliferation of perhipheral fibroblasts, creating lamellar rings of hyaline collagen, thus enhancing interstitial fibrosis and further organ damage (44).

In our experience sGIN-AKI is much more responsive to steroid treatment in cases of *fever at exordium,* also in the presence of some negative prognostic factors like severe interstitial infiltrate. Fever, present in acute onset, could be related to a greater inflammatory burst more responsive to steroids. Fever characterizes the two acute sarcoidosis syndromes with relatively benign clinical outcome: Lofgren syndrome and Heerfordt syndrome (fever, parotidomegaly, anterior uveitis, and cranial nerve palsy).

sACE was *increased* in 46%, in line with other studies: Berliner reported 50% (21/42 tested) (26), Mahevas 55% (22/41) (15), Javaud 45% (9/20) (11). The prevalence of sACE increase was similar even in other organ involvement: for instance, 36.5% in a recent study recently conducted on 74 chronic lung sarcoidosis patients in treatment for more than 1 year at Siena Regional Referral Centre for Sarcoidosis (45). sACE increase should be considered as parameter of disease burden because it is produced in granulomas, which could justify how in our survey sGIN-AKI patients with GC at RBx with sACE increase had more severe AKI, but we can't be conclusive regarding outcomes for the little sample size. We can't explain how in our experience sACE increase is rather associated with No-sGIN-AKI patients than GC granulomas. Beyond little sample size, there are other biases suggesting to be prudent with sACE increase observations: firstly, investigation was not available for all patients (sACE available in 28/36 AKI-patients); secondly, different laboratories adopted different cut-off and the laboratory method of sACE assay changed over time changing the reference values (one received sarcoidosis diagnosis in 1987); thirdly, we didn't consider the use of ACE inhibitors. Last but not least, different grade of AKI could interfere in sACE levels for different renal clearances.

Our study does not have the value of a large-scale epidemiological study, it can't estimate Italian incidence of renal sarcoidosis because it doesn't describe the total number of renal biopsies performed during the study periods, nevertheless our study collects the biggest Italian case series of renal sarcoidosis involving many important Italian centres with great experience in the field of renal pathology. Renal involvement of sarcoidosis is very rare: only 2 patients had renal involvements among 640 patients diagnosed with sarcoidosis were registered from 1976 to 2015 by Mañá in Bellvitge University Hospital, a tertiary university hospital in Barcelona (46). Another major strengths of our study is long FU. In our knowledge this is the first series of renal sarcoidosis reported in Italy and the first case series of renal sarcoidosis applying phenotypes of GenPhenReSa project. On the other hand, our weakest point is that this survey presents many referral and selection biases. Firstly, the 5 No-sGIN-AKI patients can't be well matched with 31 sGIN-AKI patients in terms of size number and clinical characteristics (but methodological rigor can hardly be applied to little sample size like this and in a rare disease like renal sarcoidosis). Secondly, there wasn't a clear indication for intravenous steroid, so patients were treated in different hospital with different clinical approaches, dosages, schedules and protocols. Indeed there wasn't a standard immunosuppressive protocol to be administered, only in 2014 a protocol for the management of sarcoidosis in different situations has been suggested (47). Thirdly, renal biopsies were not independently reviewed by a single pathologist. Lastly, there are many data loss, in line with survey considering patients treated even 20 years ago (not all data were digitized but reported in paper medical records).

Conclusion

As showed by our survey renal involvement could be the first sign of a hidden systemic sarcoidosis, that should considered in a patient with some unexplained degree of renal impairment and negative autoimmunity, also in absence of urinary abnormalities or proteinuria. Sometimes RBx could rule out the diagnosis of an unacknowledged systemic disease, leading to the discovery of other extra-renal localizations. It's fundamental characterizing the type of renal involment: above all the presence of sGIN with giant cells, high

degrees of cellular infiltrate and interstitial fibrosis are predictors of the worst long term renal prognosis. If by definition "high risk sarcoidosis" gathers severe systemic sarcoidosis at higher risk for mortality as treatment-resistant pulmonary sarcoidosis phenotypes (e.g., pulmonary fibrosis, pulmonary hypertension), cardiac sarcoidosis and neurosarcoidosis (48), similarly sGIN-AKI deserves a particular attention because it is often refractary to standard steroid therapy and can result in some degree of CKD, with related cardiovascular complications and dialysis. In this hystological patterns macrophages and T-cells accumulation must be avoided in the early stages of granuloma formation, thus preventing further kidney injuries as giant cell appearance leading to CKD. Probably the presence of giant cells granulomas, high grade of interstitial infiltrate and high grade of interstitial fibrosis suggest to be more aggressive with therapy to avoid residual CKD, using other immunosoppressants, particularly with no complete recovery at first line steroid therapy in a male patient with III stage-normocalcemic AKI and subacute onset. For instance, infliximab (49) and adalimumab (50) demonstrated promising results in pulmonary sarcoidosis, but further trials are needed in renal sarcoidosis. For mantainance therapy antifibrotic drugs to suppress subsequent fibrosis may be introduced, as previously suggested (19). Moreover, maintenance steroid therapy should be last at least 2 years (11), much more longer than commonly used and registered in our survey.

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References

1. Baughman RP, Lower EE, Du Bois RM. Sarcoidosis. Lancet 2003; 361(9363):1111-1118.
2. Adams DO. The granulomatous inflammatory response. A review. Am J Pathol 1976; 84(1):164-192.
3. Valeyre D, Nunes H, Bernaudin JF. Advanced pulmonary sarcoidosis. Curr Opin Pulm Med 2014; 20(5):488-495.
4. Calender A, Jeny F, Nunes H. Sarcoidosis [Internet]. Orphanet: The portal for rare diseases and orphan drugs. [quoted on 19 March 2021]. Available on: https://www.orpha.net/consor/cgi-bin/Disease_Search.php?lng=EN&data_id=735.
5. Tinelli C, De Silvestri A, Richeldi L, Oggionni T. The Italian register for diffuse infiltrative lung disorders (RIPID): a four-year report. Sarcoidosis Vasc Diffuse Lung Dis 2005;22:S4-8.
6. Beghè D, Dall'Asta L, Garavelli C, Pastorelli AA, Muscarella M, Saccani G, et al. Sarcoidosis in an Italian province. Prevalence and environmental risk factors. PloS one. 2017;12(5):e0176859.
7. European Commission Report. Useful information on rare diseases from an EU perspective. Retrieved 19 May 2009.
8. Brito-Zeron P, Kostov B, Superville D, Baughman RP, Ramos-Casals M. Geoepidemiological big data approach to sarcoidosis: geographical and ethnic determinants. Clin Exp Rheumatol. 2019;37(6):1052–64.
9. Schupp JC , Freitag-Wolf S , Bargagli E , et al. Phenotypes of organ involvement in sarcoidosis. Eur Respir J 2018; 51:170099.
10. Longcope WT, Freiman DG. A study of sarcoidosis; based on a combined investigation of 160 cases including 30 autopsies from The Johns Hopkins Hospital and Massachusetts General Hospital. Medicine (Baltimore) 1952; 31(1):1-132.
11. Javaud N, Belenfant X, Stirnemann J, Laederich J, Ziol M, Callard P, et al. Renal granulomatoses: a retrospective study of 40 cases and review of the literature. Medicine. 2007;86(3):170–80.
12. Bijol V, Mendez GP, Nose V, Rennke HG. Granulomatous interstitial nephritis: a clinicopathologic study of 46 cases from a single institution. International Journal of Surgical Pathology. 2006;14(1):57–63.
13. Bagnasco SM, Gottipati S, Kraus E, Alachkar N, Montgomery RA, Racusen LC, et al. Sarcoidosis in native and transplanted kidneys: incidence, pathologic findings, and clinical course. PLoS One. 2014;9(10):e110778.
14. Löffler C, Löffler U, Tuleweit A, Waldherr R, Uppenkamp M, Bergner R. Renal sarcoidosis: epidemiological and follow-up data in a cohort of 27 patients. Sarcoidosis Vasc Diffuse Lung Dis 2015; 31(4):306-315.
15. Mahévas M, Lescure FX, Boffa J-J, Delastour V, Belenfant X, Chapelon C, et al. Renal sarcoidosis: clinical, laboratory, and histologic presentation and outcome in 47 patients. Medicine. 2009;88(2):98–106.
16. Simerville JA, Maxted WC, Pahira JJ. Urinalysis: a comprehensive review. Am Fam Physician. 2005 Mar 15;71(6):1153-62. Erratum in: Am Fam Physician. 2006 Oct 1;74(7):1096. PMID: 15791892.
17. Levin A, Stevens PE. Summary of KDIGO 2012 CKD Guideline: behind the scenes, need for guidance, and a framework for moving forward. Kidney international. 2014;85(1):49–61.
18. Kdigo A. Work Group. KDIGO clinical practice guideline for acute kidney injury. Kidney Int Suppl. 2012;2(1):1–138.
19. Kamata Y, Sato H, Joh K, Tsuchiya Y, Kunugi S, Shimizu A, et al. Clinical characteristics of biopsy-proven renal sarcoidosis in Japan. Sarcoidosis Vasc Diffuse Lung Dis 2018;35(3):252.
20. Ungprasert P, Carmona EM, Crowson CS, Matteson EL. Diagnostic utility of angiotensin#converting enzyme in sarcoidosis: a population-based study. Lung. 2016;194(1):91–5.
21. Bunting PS, Szalai JP, Katic M. Diagnostic aspects of angiotensin converting enzyme in pulmonary sarcoidosis. Clin Biochem. 1987;20(3):213–9.
22. Niederer RL, Al-Janabi A, Lightman SL, Tomkins-Netzer O. Serum angiotensin-converting enzyme has a high negative predictive value in the investigation for systemic sarcoidosis. Am J Ophthalmol. 2018;194:82–7.
23. Ramos-Casals M, Retamozo S, Sisó-Almirall A, Pérez-Alvarez R, Pallarés L, Brito-Zerón P. Clinically-useful serum biomarkers for

diagnosis and prognosis of sarcoidosis. Expert Rev Clin Immunol. 2019;15(4):391–405.
24. Makris K, Spanou L. Acute Kidney Injury: Definition, Pathophysiology and Clinical Phenotypes. Clin Biochem Rev. 2016 May;37(2):85-98. PMID: 28303073; PMCID: PMC5198510.
25. Rajakariar R, Sharples E, Raftery M, Sheaff M, Yaqoob M. Sarcoid tubulo-interstitial nephritis: long-term outcome and response to corticosteroid therapy. Kid int. 2006;70(1):165–9.
26. Berliner AR, Haas M, Choi MJ. Sarcoidosis: The Nephrologist's Perspective. Am J Kid Dis 2006; 48(5): 856-870.
27. Bergner R, Hoffmann M, Waldherr R, Uppenkamp M. Frequency of kidney disease in chronic sarcoidosis. Sarcoidosis Vasc Diffuse Lung Dis 2003; 20(2):126-132.
28. Sanada S, Yoda S, Sato T. Pathological value of lysozyme staining for renal sarcoidosis. Nephrology Dialysis Transplantation. 2020;35(9):1638–41.
29. Patel TV, Rennke HG, Sloan JM, DeAngelo DJ, Charytan DM. A forgotten cause of kidney injury in chronic myelomonocytic leukemia. Am J Kid Dis 2009;54(1):159–64.
30. Iannuzzi MC, Rybicki BA, Teirstein AS. Medical progress. Sarcoidosis. N Eng J Med. 2007;357:2153–65.
31. Toriu N, Sumida K, Oguro M, Oshima Y, Mizuno H, Hasegawa E, et al. Increase of 1, 25 dihydroxyvitamin D in sarcoidosis patients with renal dysfunction. Clin Exp Nephrol. 2019;23(10):1202–10.
32. Tebben PJ, Singh RJ, Kumar R. Vitamin D-mediated hypercalcemia: mechanisms, diagnosis, and treatment. Endocr Rev. 2016; 37(5):521–47.
33. Baughman R, Janovcik J, Ray M, Sweiss N, Lower E. Calcium and vitamin D metabolism in sarcoidosis. Sarcoidosis Vasc Diffus Lung Dis. 2013; 30(2):113–20.
34. Derose SF, Rutkowski MP, Crooks PW, Shi JM, Wang JQ, Kalantar-Zadeh K, et al. Racial differences in estimated GFR decline, ESRD, and mortality in an integrated health system. Am J Kidney Dis. 2013;62(2):236–44.
35. Peralta CA, Katz R, DeBoer I, Ix J, Sarnak M, Kramer H, et al. Racial and ethnic differences in kidney function decline among persons without chronic kidney disease. J Am Soc Nephrol 2011;22(7):1327–34.
36. Baba M, Shimbo T, Horio M, Ando M, Yasuda Y, Komatsu Y, et al. Longitudinal study of the decline in renal function in healthy subjects. PLoS One. 2015;10(6):e0129036.
37. Wetzels J, Kiemeney L, Swinkels D, Willems H, Den Heijer M. Age- and gender-specific reference values of estimated GFR in Caucasians: the Nijmegen Biomedical Study. Kidney Int. 2007;72(5):632–7.
38. Koraishy FM, Hooks-Anderson D, Salas J, Rauchman M, Scherrer JF. Fast GFR decline and progression to CKD among primary care patients with preserved GFR. Int Urol Nephrol. 2018;50(3):501-508.
39. Krolewski AS, Skupien J, Rossing P, Warram JH . Fast renal decline to end-stage renal disease: an unrecognized feature of nephropathy in diabetes. Kidney Int. 2017;91(6):1300–11.
40. Zissel G, Prasse A, Muller-Quernheim J. Immunologic response of sarcoidosis. Semin Respir Crit Care Med 2010; 31(4):390-403.
41. Sakthivel P, Bruder D. Mechanism of granuloma formation in sarcoidosis. Curr Opin Hematol 2017; 24(1):59-65.
42. Ueda S, Murakami T, Ogino H, Matsuura M, Tamaki M, Kishi S, et al. 2 Systemic Sarcoidosis Presenting with Renal Involvement Caused by Various Sarcoidosis-associated Pathophysiological Conditions. Internal Medicine. 2019;58(5):679–84.
43. Mortaz E, Rezayat F, Amani D, Kiani A, Garssen J, Adcock IM, et al. The Roles of T Helper 1, T Helper 17 and Regulatory T Cells in the Pathogenesis of Sarcoidosis. Iran J Allergy Asthma Immunol. 2016; 15(4):334-339.
44. Mitchell DN, Scadding JG. Sarcoidosis. Am Rev Respir Dis 1974; 110(6):774-802.
45. Bergantini L, Bianchi F, Cameli P, Mazzei MA, Fui A, Sestini P, et al. Prognostic Biomarkers of Sarcoidosis: A Comparative Study of Serum Chitotriosidase, ACE, Lysozyme, and KL-6. Dis Markers. 2019 Mar 3; 2019:8565423. doi: 10.1155/2019/8565423. eCollection 2019.
46. Mañá J, Rubio-Rivas M, Villalba N, Marcoval J, Iriarte A, Molina-Molina M, et al. Multidisciplinary approach and long-term follow-up in a series of 640 consecutive patients with sarcoidosis Cohort study of a 40-year clinical experience at a tertiary referral center in Barcelona, Spain. Medicine 2017; 96:29(e7595).
47. Hilderson I, Van Laecke S, Wauters A, Donck J. Treatment of renal sarcoidosis: is there a guideline? Overview of the different treatment options. Nephrol Dial Transplant 2014; 29(10):1841-1847.
48. Sauer WH, Stern BJ, Baughman RP, Culver DA, Royal W. High-Risk Sarcoidosis. Current Concepts and Research Imperatives. Ann Am Thorac Soc 2017; 14(Supplement_6):S437-S444.
49. Rossman MD, Newman LS, Baughman RP, Teirstein A, Weinberger SE, Miller Jr W, et al. A double-blinded, randomized, placebo-controlled trial of infliximab in subjects with active pulmonary sarcoidosis. Sarcoidosis Vasc Diffuse Lung Dis 2006; 23(3):201-208.
50. Sweiss NJ, Noth I, Mirsaeidi M, Zhang W, Naureckas ET, Hogarth DK, et al. Efficacy Results of a 52-week Trial of Adalimumab in the Treatment of Refractory Sarcoidosis. Sarcoidosis Vasc Diffuse Lung Dis 2014; 31(1):46-54.