

# Prevalence of positive ANA, ANCA antibodies and 25(OH) vitamin D levels in patients in hemodialysis

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## Abstract

**Objective:** To describe the prevalence and factors associated with the positivity of FAN-Hep2, ANCA and 25 (OH) vitamin D deficiency in users of a hemodialysis clinic in Southern Brazil.

**Methods:** A cross-sectional study was carried out in 68 patients from a hemodialysis clinic in the Southern Santa Catarina (Southern Brazil) from August to November 2013. An interview was conducted, data collection in an electronic medical record, FAN and ANCA research by indirect immunofluorescence WAMA and dosage of 25 (OH) vitamin D by chemiluminescence amplified in heparinized plasma.

**Results:** The prevalence of ANCA in a sample titrated 1:10 was 24.4%, with 10.3% presenting c-ANCA pattern and 14.1% presenting a p-ANCA pattern, of the total. The prevalence of ANA was 4.4%, and the prevalence of 25 (OH) vitamin D deficiency was 35.8%. No associations with clinical and sociodemographic characteristics were observed with autoantibodies.

**Conclusion:** We found a high prevalence of ANCA positive and 25 (OH) vitamin D deficiency. We emphasize the need for further studies in this group of patients to define the contribution of these tests to the diagnosis and prognosis of renal disease and its complications.

## Introduction

According to data from the World Health Organization, an estimated 500,000,000 people are chronically infected with hepatitis B virus (HBV) or hepatitis C virus (HCV) worldwide. Due to these infections and their complications, such as cirrhosis and hepatic carcinoma, approximately 1,000,000 people die annually [1]. An important route of contamination by HBV and HCV viruses is the parenteral route. Blood transfusion, surgical procedures and hemodialysis are recognized as risk factors for these infections [2,3].

Extra-hepatic manifestations have been related to the presence of chronic hepatitis, especially with the presence of HCV. Autoimmune diseases are included in these extrahepatic manifestations, due to the induction of apoptosis by T cells, by mechanisms that involve the recognition of autoantigens [4]. Renal function during liver disease has also been the focus of recent research. Decreased peripheral vascular resistance promoting renal ischemia, renal damage in the tubular portion by elevated levels of hepatic metabolites, especially in cholestatic diseases, and deposition of immunocomplexes in the glomerular portion are the main mechanisms that explain the pathophysiological relationship between the kidney and liver [5].

Antinuclear Antibody (ANA) screening by indirect immunofluorescence is a sensitive and useful assay for screening of autoimmune diseases. The application and interpretation of the test must be judicious and confirmed by the search for specific autoantibodies through other techniques, such as ELISA and Western blot [6]. Antibody search for neutrophils (ANCA), however, provides a useful tool in the investigation of vasculitis [7]. Renal involvement

in vasculitis associated with ANCA positivity is a frequent finding in clinical practice, and the presence of this marker is a negative indicator of prognosis [8].

It is also known that patients with renal disease have high frequency of 25 (OH) vitamin D deficiency, as well as, difficulty in activating this vitamin [9-11]. However, 25 (OH) vitamin D deficiency causes damage not only to bone metabolism, but systemic, since it is a source of immune system modulation. Clinical evidence corroborates this fact, through the relationship between vitamin D 25 (OH) deficiency and increased frequency of various autoimmune diseases [12].

Considering that the metabolism of vitamin D depends on hepatic and renal health, and the complexity of the pathophysiological mechanisms that relate the triad "autoimmunity - renal disease - liver disease", there is a need for studies that provide support to improve diagnosis and understanding of the clinical evolution of these diseases. The goal of this study is to characterize a group of patients with renal insufficiency regarding clinical aspects and to investigate the prevalence of autoantibodies by FAN, ANCA and 25 (OH) vitamin D. The

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identification of factors associated with the presence of these markers may contribute to refining the application and interpretation of the same in clinical practice.

The aim of this study was to describe the prevalence and factors associated with the positivity of FAN-Hep2, ANCA and 25 (OH) vitamin D deficiency in users of a hemodialysis clinic in Southern Brazil and associated factors.

## Methods

A cross-sectional study was conducted, which census of the population of the Kidney Disease Clinic of the city of Tubarão – Santa Catarina, Southern Brazil, from August to November 2013. Patients with renal insufficiency undergoing hemodialysis treatment were included, who underwent periodic exams in the Laboratory of Clinical Analysis of the University of Southern Santa Catarina (LAC-UNISUL), aged 18 years or older. Exclusion criteria were: patient refusal to study, patients with cognitive impairment, previously diagnosed autoimmune diseases (lupus, rheumatoid arthritis, thyroiditis, etc.), and patients absent from hemodialysis sessions on at least three previously scheduled visits. This work was approved by the Research Ethics Committee (CEP) of UNISUL, protocol number 338.734.

Data collection was initiated with invitation and semi-structured questionnaire application. Dosing of 25 (OH) vitamin D was performed by amplified chemiluminescence (Ortho Clinical Diagnostics Johnson and Johnson) in heparinized plasma samples of these patients. The FAN and ANCA research by indirect immunofluorescence (WAMA diagnosis) was performed in heparinized plasma obtained from the Laboratory of Clinical Analysis of UNISUL.

Statistical analysis was done with SPSS software version 20.0. The mean, median and standard deviation for the continuous variables and proportions for the categorical variables were calculated. For the test of association between the variables of interest, Pearson's chi-square test and Fisher's exact test were used, when appropriate. The pre-established level of significance was 95%.

## Results

From the 127 patients treated by the Renal Disease Clinic, 101 patients had collected heparinized plasma for periodic examinations by the LAC-UNISUL at the time of the study and were previously included in the study. Twelve losses occurred: two refusals, three deaths, two discharges, one patient with cognitive impairment and four absences at hemodialysis sessions. Of the remaining 89 participants, ten patients were lost due to lack of exams and eleven autoimmune diseases were excluded from the study: lupus (1), rheumatoid arthritis (3), vasculitis and thyroiditis (4) (Tables 1 and 2).

From the 32 patients who reported receiving a blood transfusion, 14 (20.6%) reported having received at least one prior to 1993. From the 68 patients, only 3 (4.4%) reported having been drinking alcoholic beverages socially. No patient had Hepatitis B infection and three patients (4.4%) had Hepatitis C.

In relation to ANA, 4.4% of the sample were positive. For ANCA antibodies, 51 (75.0%) patients had non-reagent results, and 17 (24.4%) had reagents. About ANCA reagents, 6 (8.8%) presented c-ANCA and 11 (16.2%) presented p-ANCA pattern (Table 3).

The female patients presented levels of 25 (OH) vitamin D lower than those of the male sex did ( $p=0.004$ ). Patients with SD also had

**Table 1.** Sociodemographic Characteristics

Sociodemographic Characteristics	N	%
Gender		
Female	25	36.8
Male	43	63.2
Race		
White	57	83.8
Black	11	16.2
Age (years)		
21-40	8	11.9
41-50	15	22.0
51-70	36	52.9
71-80	7	10.3
>80	2	2.9
Age (Mean $\pm$ standard deviation in years)	Mean=55.94	SD=14.79
Scholarity (completed)		
0-4	23	33.8
5-8	27	39.7
9-11	14	20.6
12-16	4	5.9
Scholarity (mean $\pm$ standard deviation in years)	mean=6.81	SD=3.84

**Table 2.** Clinical characteristics of the patients

Clinical characteristics	N	%
Comorbidity		
Arterial hypertension (HAS)	48	70.6
Diabetes Mellitus (DM)	31	45.6
Urinary infection of repetition	9	13.2
Kidney stone	6	8.8
Dyslipidemia	28	41.2
Hepatitis C	3	4.4
Hepatitis B	0	0
Transfusion		
Never received	36	52.9
Received until 3 times	28	41.1
Received from 4 to 6 times	1	1.5
Received from 7 to 10 times	1	1.5
Received more than 10 times	2	3.0
Hemodialysis' time (in months)		
1 – 24 months	43	63.2
25-50 months	10	14.7
51-80 months	7	10.3
81-100 months	2	3.0
101-180 months	6	8.8
Mean and standard deviation (SD) Primary causes of kidney injury	mean=35.96	SD=38.92
Routine tests or doesn't know the fact that related to the diagnostic of the kidney injury	27	39.7
Symptoms (pain, edema, weakness)	12	17.6
Hypertension	9	13.2
Infection of urinary tract	2	2.9
Diabetes	7	10.3
Vascular accident (angina, heart attack, stroke)	4	5.8
Hereditary / congenital	2	2.9
Kidney stone	2	2.9
Drug toxicity	3	4.4
Creatinine (pre-dialysis)	mean=9.93	SD=2.64
Urea	mean=156.44	SD=37.12
Platelets	mean=191313.24	SD=22.44

**Table 3.** Prevalence of positivity of ANA, ANCA, and levels of 25 (OH) vitamin D

Variable	N	%
<b>ANA</b>		
positive	3	4.4
negative	65	95.6
<b>ANCA</b>		
negative	51	75.0
mean cytoplasmic (c-ANCA)	6	8.8
Mean perinuclear (p-ANCA)	11	16.2
<b>25 (OH) Vitamin D (25 OH cholecalciferol)</b>		
0-10	0	0
11-20	6	8.8
21-30	18	26.5
>30	44	64.7
<b>25 (OH) Vitamin D (mean and standard deviation)</b>	mean=38.87	SD=13.07

**Table 4.** Comparison of 25 (OH) vitamin D levels among the variable sociodemographic, clinical and laboratorial tests

Variable	Mean	Standard deviation	p-value
<b>Gender</b>			0.004*
Male	43.14	16.61	
Female	31.54	12.50	
<b>Race</b>			0.410*
White	35.17	12.18	
Non white	39.59	16.80	
<b>Diabetes mellitus</b>			
Yes	36.76	18.44	0.016*
No	43.13	12.67	
<b>Arterial hypertension</b>			0.439*
Yes	37.89	17.37	
No	41.25	12.83	
<b>Corticoids use</b>			0.611*
Yes	33.10	18.10	
No	39.05	16.21	
<b>ANA</b>			0.875*
Positive	38.81	16.37	
Negative	40.33	12.29	
<b>ANCA</b>			0.231*
Negative	40.08	16.39	
Positive	35.27	13.28	

\*p&lt;0.05 – Chi square test

lower levels of 25 (OH) vitamin D when compared to those without SD (p=0.016) (Table 4).

There were no associations between the levels of 25 (OH) vitamin D and the ANA (p=0.875) and ANCA (p=0.321) levels. The clinical or sociodemographic characteristics also did not present a statistically significant association with the positivity of ANA and ANCA antibodies.

## Discussion

Socio-demographic characteristics found in this study (sex, age, race and schooling) are in line with the Brazilian reality of patients with chronic kidney disease and with the European colonization of Santa Catarina [13,14]. Low schooling also reduces the patient's chances of understanding and adhering to medical recommendations, as well as giving an opinion on managing their illness.

The high prevalence of patients undergoing blood transfusion is a reflection of the socio-demographic profile of these patients. Due to the risk for HBV and HCV infection, as well as for the development

of irregular antibodies against erythrocyte antigens and other autoantigens promoted by blood transfusion and/or blood derivatives, especially prior to 1993 when screening for HCV was not yet performed in donors, the recommendations for this procedure are quite discerning [15,16]. However, no statistical correlation was found between blood transfusion and autoimmunity markers.

Patients' reports regarding the etiology of kidney disease were inconclusive because 39.7% did not know how to answer this question. However, these data are not discrepant with the data of comorbidities, suggesting that, in the majority of patients, renal disease secondary to a previous metabolic disease.

As for the comorbidities reported by the patients, systemic arterial hypertension, diabetes mellitus and dyslipidemia were the most frequent, which has also been reported in other Brazilian studies [17,18]. Interestingly, the pathophysiology of renal disease is intertwined with the pathophysiology of these three entities, the main points being the deterioration of the glomerular barrier with loss of proteins and lipoproteins, and the renin-angiotensin and hepatic system reflex with increased synthesis of acute phase proteins and lipoproteins [19].

According to the recent positioning of the Brazilian Society of Clinical Pathology and Laboratory Medicine, and the Brazilian Society of Endocrinology and Metabolism, the reference range for dosing 25 (OH) vitamin D in patients with chronic kidney disease is between 30 and 60 ng/mL [20]. In this study, 64.7% of the patients had serum 25 (OH) vitamin D levels higher than 30 ng / mL, reflecting an important prevalence of 25 (OH) vitamin D deficiency [20]. At this point, it is interesting to note that the receptor for 25 (OH) vitamin D is present not only in hard tissues and parathyroid, but also in pancreatic islets, and that there is a clinical correlation between 25 (OH) vitamin D deficiency, obesity and risk of developing diabetes mellitus [21,22].

The detection of VDR in immunological cells such as macrophages, dendritic cells and T and B lymphocytes has also encouraged research that attempts to elucidate the role of 25 (OH) vitamin D in immunomodulation. In clinical practice, 25 (OH) vitamin D deficiency expresses a positive correlation with several autoimmune diseases [23]. It is known, however, that the occurrence of autoantibodies such as ANA and ANCA is a result of a multifactorial process, influenced by intrinsic factors such as immunomodulation and polymorphisms of histocompatibility molecules, and extrinsic factors such as viral, bacterial and alloantigen exposure and to drugs [24,25]. However, in our study, there was no association among 25 (OH) vitamin D deficiency, blood transfusion, hepatitis C infection, use of corticosteroids or interferon, and autoimmunity markers.

The ANA positivity, according to the standard and titration, can contribute to the diagnosis of several autoimmune conditions, whereas the ANCA is related to the diagnosis of three systemic necrotizing small vessel vasculitis: Microscopic Polyangiitis and its renal variant (Glomerulonephritis and Churg-Strauss syndrome and Wegener's granulomatosis [26,27]. The presence of these antibodies may promote the development or progression of renal disease by immune complexation and activation of the complement system [19]. We consider that this topic requires further studies, which include dilutions other than those recommended in the package inserts, to optimize the clinical interpretation of these autoantibodies and their predictive capacity.

## Conclusion

This study found no statistically significant correlation between ANCA, ANA, 25 (OH) vitamin D and laboratory markers of

advanced chronic kidney disease, or clinical and socio-demographic aspects of these patients. However, this topic requires more studies, since the literature offers sufficient subsidence to propose that these autoantibodies participate actively in the pathophysiology of renal disease and may occur as a cause of kidney disease or in its natural course.

## Conflict of interests

The authors declare no conflicts of interest.

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