"OVIDIUS" UNIVERSITY OF CONSTANTA
DOCTORAL SCHOOL OF MEDICINE
FIELD: MEDICINE DOCTORAL STUDIES

SUMMARY
OF THE DOCTORAL THESIS

Scientific Coordinator:
Prof.Univ.Dr. RUGINĂ SORIN

PhD student:
Şotilă (Ivan) Gianina Gabriela

CONSTANTA
2020

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EARLY ASSESSMENT MARKERS OF RENAL DYSFUNCTION IN HIV-AIDS INFECTION

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The doctoral thesis is structured in two parts:

- **Part I: Current state of knowledge**, organized in 8 eight chapters
- **Part II: Personal contributions**, organized in 8 eight chapters
- Bibliographical references
- 48 Tables
- 159 Images
- Abbreviations

KEYWORDS: renal dysfunction, HIV Infection, eGFR CKD-EPI, eGFR MDRD, predictive factors of chronic kidney disease in HIV infection, HIV-1-F viral subtype, medical imaging in HIV infection

NOTE: The tables, images, abbreviations, bibliographical references and summary inserted in the present summary maintain the original numbering from the original doctoral thesis. The table of contents represents the original table of contents of the thesis.

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List of abbreviations

ACR Albuminuria/creatinuria ratio

ADQI Group Acute Dialysis Quality Improvement Initiative

AIDS Acquired immunodeficiency syndrome

NAID Nonsteroidal Anti-Inflammatory drug

AKI Acute kidney injury

AKIN Acute kidney injury network

DNA Dezoxiribonucleic acid

RNA Ribonucleic acid

mRNA messenger RNA

cARV Combined antiretroviral therapy

ATV Atazanavir

ATV-r Atazanavir boosted with ritonavir

CKD Chronic kidney disease

ESRD End stage chronic kidney disease

BUN Ureea Nitrogen

CD4 Lymphocytes CD4

CDC Center for Infectious Disease Control

CIC Circulating immune complexes

CMV Cytomegalovirus

CNLAS Anti-AIDS Committee

Cr Creatinine

CRFs Circulating recombined forms

ClCr Clearance of creatinine

CysC Cystatine C

CKD Chronic kidney disease

CKD-EPI Chronic kidney disease - epidemiology collaboration equation

CCR5 CCR5 co-receptors

CCR5 inhibitors CCR5 inhibitors

DEXA Bone densitometry test

DRV Darunavir

DSV Descovy (emtricitabine and fumarate tenofovir alafenamine)

DU Urinary flow

DM Diabetes mellitus

EACS European AIDS Clinical Society

eClCr estimated Clearance of Creatinine

eGFR estimated glomerular filtration rate

EI Entry inhibitors

EMEA European Medicines Agency

ELISA Enzyme linked immunoasorbent assay

ESRD end stage renal disease

FDA U.S. Food and Drug Administration

FTC Emtricitabina

GNV Genvoya (elvitegravir, cobicistat, emtricitabina and tenofovir alafenamine fumarate)

HAART Highly active antiretroviral therapy

H.iPTH intact Parathyroid hormone

HIV-RNA HIV Viraemia

AHT Arterial hypertension

HIV Human immunodeficiency virus

HIVAN HIV associated nephropathy

HIVICK - HIV immune complex disease

HIV-FGS – focal segmental glomerulosclerosis associated to HIV

HUS Hemolytic uremic syndrome

IECA Angiotensin converting enzyme inhibitors

IgA Immunoglobulin A

IgM Immunoglobulin M

IgG Immunoglobulin G

IL 18 Interleukine 18

NNRTI Non nucleoside reverse transcriptase inhibitors

NRTI Nucleosidic reverse transcriptase inhibitors

IN Integrase Inhibitors

PI Protease inhibitors

PI/r Boosted protease inhibitors

ARF Acute renal failure

CRF Chronic renal failure

UTI Urinary tract infection

IDV Indinavir

IDVr Boosted Indinavir

KDIGO Kidney Disease Improving Global Outcomes Clinical Practice Guideline

LAR Acute kidney injury

LPV Lopinavir

MDRD Modification of diet in renal disease study equation

MRP 2 Multidrug resistance protein 2

MRP 4 Multidrug resistance protein 4

MSM Men having sexual relations with men

PNL Percutaneous nephrolithotomy

ATN Acute tubular necrosis

ATIN Acute tubular – interstitial necrosis

OAT1 Organic anion transporter1

PBMC Peripheral blood mononuclear cells

PBR Kidney biopsy

PCR Polymerisation chain reaction

PLWH People living with HIV

PrEP Pre-exposure prophylaxis

PRT Proximal renal tubulopathy

RIFLE (risk, injury, failure, loss, end stage renal disease) Classification criteria for acute renal failure

RTV Ritonavir

AIDS Acquired immunodeficiency syndrome

SQV Saquinavir

TAF Tenofovir alafenamide fumarate

TB Tuberculosis

TDF Tenofovir fumarat**e**

UNAIDS The Joint United Nations Programme on HIV/AIDS

URFs Unique recombined forms

Uro-CT Urography by computer tomograph

USRDS The United States Renal Data System

VHB Hepatitis virus B

VHC Hepatitis virus C

VHD Hepatitis virus D

WHO World Health Organisation

INTRODUCTION

At the end of 2019, 1064 patients were registered in the Regional Center for HIV – AIDS infection Surveillance and Monitoring of Constanta, under the National Agency for the Control of AIDS, a center including the Constanta and Tulcea counties, most of the patients originating from Constanta county, with more that 500 patients from the pediatric cohort. In Romania, according to the Compartment for HIV/AIDS infection Monitoring and Assessment from INBI [The National Institute for Infectious Diseases "Prof. Dr. M. Balș"] in Constanta county, the number of new patients suffering from HIV-1 infection has been, in 2019, of 72 people. Out of the total number of seropositive patients, 979 patients benefit from combined antiretroviral therapy. These patients need constant monitoring and the insurance of the TARV therapy continuity, maintaing an adherence to the treatment, identification of those in therapeutic failure and often modification of the antiretroviral treatment schemes, early identification of the adverse reaction secondary to antiretroviral therapy, especially renal secondary effects. The team following up and carring for these patients should be multidisciplinary, in order to be able to cover a large spectrum of impairment characteristic for HIV infection, a versatile impairment, representing a notable public health problem, at the level of the whole county.

The idea of this research theme has come naturally, from the observation of the current clinical practice, due to the fact that during the last years we have noted a significant number of renal dysfunction in PLWH. The renal dysfunction consecutive to the infectious diseases comprises a varied spectre clinical, biological, histopathological manifestations, depending on some of the characteristics of the host, as well as from the infecting pathological agent. All the structural components of the renal parenchima (glomeruli, tubi, insterstitium, sanguine vessels) can be the target of direct of indirect renal effects of infectious diseases, particularly HIV. Renal impairment can appear in any stage of HIV infection and it is extremely varied.

The characteristics and particulars of the HIV virus as well as its ability to infect the immunological system, forming cell and tissue sanctuaries especially in the kidney and brain does not allow cure at present but only a continuous treatment for the time being, for the whole life, reason for which the HIV infection has become, from a life-threatening infection, a chronic infection that can be controlled through antiretroviral therapy. The kidney has a fundamental and defining role in this process, representing both a reservoir of HIV -1 infection, suffering injuries through cytopathic effect of the HIV virus at the level of

the kidney podocytes, cells that are deemed as the reservoirs of the HIV virus, as well as a secondary injured organ, due to the antiretroviral medication recoverability is associated with the HIV infection.

The progress of the antiretroviral therapy, with the continuous development of new molecules in a relatively short amount of time, constraints and amplified by the dramatic evolution of the HIV pandemic and by the increased mortality rate, is one of the most dynamic domains in the history of medicine, a domain which takes up numerous material in human resources. It's not surprising that the ARV medication is extremely costly, but it is also a correct and efficient medication in terms of immunology, which determines good clinical status of the PLWH, offering considerable prolongation of life, but also in tailing the appearance of numerous chronic impairments connected with long-term toxicity of the ARV medication, especially reno toxicity, that can notably affect the quality of life of these patients.The easier access to the new, potent antiretroviral therapies which have led to the maintenance of an efficient and durable viral suppression, decrease of mortality and increase of the survival period of PLWH, has led to the appearance of an "accelerated ageing syndrome", characterised by a new and varied spectrum of diverse manifestations, affecting in many organs: kidneys, bones, neurological system, liver, cardiovascular system. As a result, the care of the seropositive patient must consider the renal comorbidities, as well as the correct management of these complications.

The kidney dysfunction of the HIV-infected patients is increasing on the world wide level, being highly prevalent, but still variable according to various international clinical studies of America, Africa, Europe and Asia, the CKD representing one of the most important comorbidities of PLWH worldwide. Proteinuria, dyslipidaemia, diabetes mellitus, arterial hypertension, cardiovascular diseases, smoking, race, the low social and economic status, the use of injectable drugs, the glomerular diseases, the mitochondrial toxicity, chronic residual pro inflammatory status, a high viraemia, lower lymphocyte CD4 level in naive or non-adherent to treatment seropositive subjects represent important comorbidities. Due to the increase of the prevalence of chronic kidney disease in HIV infection, especially during the modern era of anti-retroviral therapy, it has become a perpetual challenge and preoccupation of the international and national forums to develop screening, prevention and management strategies of the CKD in these patients. Thus, the research topic stemmed from the fact that the renal dysfunction secondary to HIV infection is highly underdiagnosed and neglected. The speciality literature in Romania has little to none studies assessing the renal function within the acquired immunodeficiency syndrome, the present thesis having as an aim to investigate these complex interactions, for the purpose of setting the methods of

detection, prevention, delay or even stop the progression of renal dysfunction and to design a prototype of the HIV-infected patient also suffering from renal dysfunction.

Motivation and objectives of the research

The chronic kidney disease in HIV infection represents a major public health problem, on an international level. The topic of the present doctoral thesis encompasses an interdisciplinary field, an approach embraced by more and more professionals by the day, namely, finding new methods, means, techniques and technologies for the early detection and treatment of the renal dysfunction in PLWH.

The idea of this research team has come naturally, due to the fact that during the last years we can note an increase of the number of renal dysfunction in seropositive subjects, there is a direct effect of the HIV virus, or as a secondary toxic effect of the antiretroviral medication, or as a secondary effect of the multiple comorbidities of the HIV infected patient, whose life span has significantly increased in the past decade, due to the use of highly efficient therapies.

In spite of the fact that the lifespan of PLWH has extended, to the point of being similar to that of the non-HIV patient, and early ageing of the HIV patient has been registered, especially of those patients included in the paediatric cohort, involving multiple comorbidities (kidney, bones cardiovascularly, endocrinological, etc).

The antiretroviral therapy has marked a major change of vision over the management of the HIV infected patient, modeling, throughout time a certain profile of the subject whose lifespan has increased and improved considerably. During the past years, we have seen '*'The radical change of how the HIV is managed, from the reduction of the immediate demise risk of the PLWH to an increase of life quality and prevention of associated comorbidities, connected both with the HIV infection per se, as well as with the co-infections, comorbidities determined by the age or by the antiretroviral medication''*.

During the past years, we have seen an increase of the prevalence of the metabolic syndrome, obesity, arterial hypertension, diabetes mellitus, cerebrovascular diseases, both in older population and in young adults, the consequence being an accentuated increase of chronic kidney disease prevalence. This epidemiological trend is becoming more and more frequent also in HIV infected patients.

The price of the survival of multiexperienced HIV infected patients, benefiting from a long therapeutic history, most of them part of the paediatric cohort ("young by age, old by treatment) has been

represented by the occurrence of a vast and varied spectrum of ARV therapy adverse reactions. The survival of quality of life of PLWH subject to antiretroviral treatment are still seriously affected by the acute and long-term complications of these therapeutical schemes, an important percentage of the patients developing end stage renal disease, resulting in the need for renal function supplementing, dialysis or a kidney transplant. As a result it is of paramount importance that the management of the renal dysfunction be improved in the seropositive patient in order to maintain a state of health and quality of life of these subjects, through a multidisciplinary care. The nephrologist should be a part of the team caring for the seropositive patient. The multidisciplinary approach of PLWH should be the rule, not the exception!

Due to the fact that within the Regional HIV - AIDS Centre of Constanta the seropositive subjects are treated using a complete and multidisciplinary method, has given me the possibility of taking contact with the magnitude of the HIV phenomenon, to assess or treat naive or multiexperienced patients, to set the possible kidney risks given by the renal injury, either acute or chronic. I have discovered a varied range of renal dysfunction, burdened by insufficient clinical data in the specialty literature, given the non-homogeneity of the studied groups and of the particulars encompassing both demographical data like race, and features connected with the HIV virus, like the viral subtypes. Few answers...Controversial data...

We have tried to identify a kidney prototype of PLWH, especially of those from the paediatric cohort, for the purpose of finding therapeutical solutions for slowing down the process of gradual loss of the renal function. Failure to diagnose in due time CKD is burdened by numerous complications, costly therapies, increased admission rates, high mortality, unsustainable costs.

The investigation and assessment of the seropositive patients by means of this doctoral research, would be the first such approach in Constanta county and on a national level.

In the context of what was previously stated, this research represents a form of study - perfectible, by all means, and aims are representing the starting point, so useful for those who in the future, should dedicate their passion and interest to this dynamic, captivating domain.

Scope and objectives of the research

Scope of the research

Considering all aspects presented in the general part, the present work aims at exposing the personal experience in the field of renal dysfunction assessment in the HIV – infection, on the studied groups.

The research topic envisages the early detection of renal injury and the anticipated effects of this process, contributing to the optimisation of prevention and the delay of the CKD progression in the seropositive patient, one of the most important objectives of modern public health policies.

Objectives of the research

Through this research project, I have aimed at attaining the following objectives:

Primary objectives:

1. The comparison of two methods of estimating the glomerular filtration rate: CKD-EPI and MDRD in a group of HIV infected patients, using combined antiretroviral therapy and the estimation of the risk of early onset of CKD in these patients.

2. To identify and assess the traditional and non-traditional risk factors (Predicting variables) for the development and progression of CKD for a group of highly experienced HIV infected patient followed up during five years, in order to gain applicability in the current practice, with important consequences over the therapeutic and interventional strategies give the prognosis, quality of life and lifespan ff these patients.

Secondary objectives:

1. Assessment of the chronic renal dysfunction in a group of patients with HIV infection and viral subtype F1, followed up during five years, using the comparison of sensitivity and specificity of two methods of estimated glomerular filtration rate: CKD-EPI and MDRD.
2. Assessment of other types of renal impairment apart from CKD in a group of highly experienced seropositive patients followed up during five years.

Method of the research. Materials and methods

Study plan

The study carried out throughout the doctoral program has taken place in the period 2014-2019 and has included 68 patients.

The research theme has involved a retrospective and prospective, observational, noninterventional study on human subjects (patients) which has included minimum invasive investigations (blood sampling, sembling in various biological cultures like vagina, urethra, urine culture), invasive imagering investigations (URO-CT, Angio-CT, renal scintigraphy, parathyroid scintigraphy) or non-invasive imagering investigations (renal ultrasound, thyroid ultrasound).The protocol did not include administrating any drugs throughout the study.

The population group

The patients diagnosed and confirmed with HIV infection were included in the study, registered with the Clinical Hospital for Infectious Diseases of Constanta, through the Day Clinic, which has subsequently become the Regional Center for HIV – AIDS Infection Surveillance and Monitoring of Constanta, or the Regional Centre for Excellence in HIV – AIDS. Over time, the Clinical Hospital Infections Diseases of Constanta has included various partnership with various Institutions from abroad or from the country. One of these partnership is it a strategic partnership with the Foundation Baylor College of Medicine and Texas Children Hospital, initiated in 2001, as well as with the Abbott Fund, partnership that head is a purpose the access of the seropositive patient to antiretroviral medication through an extended access program („expanded –access''). The beneficiaries of this type of program have been especially the patients from the paediatric cohort, which have received antiretroviral medication schemes such as lopinavir boosted with ritonavir, didanozine (ddI), ritonavir, stavudine (d4T), well-tolerated, efficient, improving the immunological status of the patients, with a high rate of viral non-detectability.

In Romania, all the patients with HIV infection have free access to the National Program against HIV-AIDS. Some of the seropositive patients from the whole experience category, have also benefited throughout time from Clinical studies, receiving unavailable antiretroviral medication through the National Program, which led to the attainment of the virus suppression and of the nondetectable status, with a remarkable increase of the quality of life.

Patient recruitment

From the whole range registered with the HIV infection Monitoring and Surveillance Centre of Constanta county, subordinated to the National Committee against HIV-AIDS, 68 patients have been registered, who have accepted to be assessed and followed up through a period of five years, from January 2014 until December 2019. The main analysed group comprises 68 patients, from which several subgroups have been derived, based on the approached topics.

Ethical considerations in the form expressing the informed consent of the patient, according to the GDPR

Scope and objectives of the research have been exposed to the Ethical Committee of the Clinical Hospital of Infectious Diseases of Constanta, who have expressed their consent and approved the study according to the legal framework in force, in connection with the necessary steps and how the study should be carried out. The Objectives of the study have been explained to the patients that have excepted voluntarily to take part in the study, exposure to possible risks as well as the benefits of participating. Subsequently, after clearing these legislative aspects, they have signed the participation consent and the informed consent forms.

The medical evaluation sheet of the subjects included in the research

The data obtained from the past medical history of the subjects included in the study had heaven written down in the medical sheets which have been subsequently introduced in a database. The medical sheets have included the following data:

- Demographical data (date and year of birth, age, environment, gender, race or ethnicity)

- Information referring to factors connected to their lifestyle, such as drug use, alcohol consumption, synthetic creatinine consumption or smoker status

– Data related to the HIV viral infection: later related to the year when the patient was positively diagnosed, data in connection with how old the infection is, staging of the impairment according to the CDC classification, how the infection was transmitted, the viral subtype, the level of the RNA HIV viraemia, the level of the CD4 lymphocytes upon discovery ofthe HIV infection, the level of the CD4 Nadir lymphocytes, the level of the lymphocytes upon entering into the study, the current antiretroviral medication and the time of exposure to the present scheme, the number of the treatment scheme previously used and their respective exposure time

- Data referring to the coinfections with the hepatitis virus B, D, C, tuberculosis either sequelae or active

– Data referring to HIV infection comorbidities: diabetes mellitus, diabetic nephropathy, arterial hypertension, hypertensive nephroangiosclerosis, cardiovascular disease, dyslipidaemia, liver steatosis, neoplasia

The seropositive patients who have accepted to voluntarily participate in the study have been assessed two times a year, six months apart between the visits. Visit 1 or moment 1, has been considered the first evaluation, when the personal, demographical and medical data of the patients have been filled in the inclusion study. At this time, the lab analysis have been sampled and the abdominal and pelvic ultrasound has been performed. The biological samples coming from the patients included in the research have been performed in the Lab of the Clinical Hospital for Infectious Diseases of Constanta. In the study group, comprising 68 seropositive patients, the glomerular filtration rates have been calculated at least twice a year, in the period 2014-2019, using the two calculus methods CKD-EPI and MDRD, taking into account of the four variables: serum creatinine, age, gender, race. The patients have been followed up dynamically, being monitored most from their point of view of their virological, immune and kidney evolution, entering the biological and clinical point of view.

Inclusion and exclusion criteria

The criteria for being included in the study have been as follows:

- Age above 18 years
- Criteria of HIV viral infection present
- Signing the informed consent form, necessary for inclusion in the study.
- Patients with the glomerular filtration rate $\geq$ 60 ml/min/1,73m² assessed through the two methods: CKD-EPI and MDRD

The exclusion criteria have been as follows:

- Patient on dialysis
- Patient with a transplant
- Patient with a eGFR lower than 60 ml/min/1,73m² upon registration

Case definitions

The cART medication has been defined as the use of at least 2 drugs from two different classes, capable of insuring viral suppression.

Definition of the CKD and the staging of the CKD has been performed according to the KDOQI guide.

- $\geq$ 90 or above 90 ml/min/1,73m²- stage 1 (normal or near normal kidney function)

- 60 to 89 ml/min/1,73m²-stage 2 (mild loss of kidney function)
- 30 to 59 ml/min/1,73m²-stage 3
- 45 to 59 ml/min/1,73m²- stage 3a (mild to moderate loss of kidney function)
- 30 to 44 ml/min/1,73m2-stage 3b (moderate to severe loss of kidney function)
- 15 to 29 ml/min/1,73m²-stage 4 (severe loss of kidney function)

Under the conditions in which the glomerular fixation rate is below or equal to 60 ml/min/1,73m² for a period of at least three months, it is deemed that the chronic renal disease exists, even in the absence or presence of renal injury.

Diabetes mellitus has been defined as administering antidiabetic medication by oral intake or is insulin or its confirmation via diabetologist based on glycaemia and glycosylated haemoglobin, prior to the patient's entry into the study.

Arterial hypertension has been defined as the use of antihypertensive medication or the confirmation of the diagnosis by a cardiologist (systolic blood pressure ≥ 140mmHg, diastolic blood pressure ≥ 90mmHg), prior to the patient's entry into the study

Cardiovascular diseases have been defined as a presence in the history of the patient prior to entering into the study of the following medical conditions: congestive cardiac failure, myocardial infarction, cerebral vascular attack, coronary angioplasty and stents, carotid endarterectomy, coronary bypass surgery, peripheral vascular disease.

Metabolic syndrome has been defined as the presence in the same individual of at least three of the following entities: central type obesity, defined as the increase of the abdominal circumference above 102 cm in men and about 88 cm in women, altered tolerance to glucoses - defined through slightly increased values of the glycaemia (between 110-126 mg/dl-a jeun), triglyceride increase above 150 mg/dl, decrease of HDL cholesterol below 40 mg/dl in men and below 50 mg /dl in women, increase of the values of arterial blood pressure above 130/85 mmHg.

Dyslipidaemia has been defined as the use of hypolipidaemic drugs (statins or fibrates) prior to entering into the study.

Viral hepatitis B has been defined as the presence of the antigen HBs or DNA VHB detectable viremia. Viral hepatitis D has been defined as the presence of the antigen VHD or RNA VHD viremia.

Viral hepatitis C has been defined as the presence of the antibodies anti-HCV or RNA VHC detectable viremia.

Tuberculosis (TB) sequellae has been defined as the presence of the TB sequellae in a patient with a history of active TB that has undergone a tuberculostatic treatment. The active tuberculosis has been defined at the presence of positive cultures for BK, current anti-tuberculostatic treatment confirmed by a pneumologist.

Proteinuria has been defined as the presence in the urinalysis of at least 30 mg/dl proteins. For the patients confirmed with the proteinuria in the year analysis, the value for proteinuria has been checked for 24 hour urine protein test, as well as the ratio albuminuria/creatinuria.

Haemoglobin, red-cell indexes and blood iron, have been measured for all the subjects of the study. The anaemia has been defined as the value of the haemoglobin below 11.7 mg/dl.

Methods.

Lab analysis

The dynamic determination of the medical analysis has been performed upon registration and every six months for the period of the 60 month of follow-up (2014-2019).

Biological samples have been taken from all patients in order to check the values of interest:

- **Blood samples**: hemogram [haemoglobin (Hgb), regular values –11,7-15,5 g/dl, HCT, regular values 35-45%, MCV- 81-100/fL, HEM, regular values 27-34 pg, CHEM, regular values 32-36 g/dl].
- **Serum biochemistry in order to assess the lipidic profile** [cholesterol seric total, regular values 110-200 mg/dL, HDL cholesterol-high-density lipidic fractions, regular values 35-88 mg/dl LDL cholesterol-low density fractions, regular values 40-140 mg/dl, serum triglycerides (TGL) regular values 50-165 mg/dl].
- **Biochemistry samples in order to assess the renal markers and the bone profile** (serum creatinine regular values 0.6-0.9 mg/dl, serum urea -15-45 mg/dl, serum uric acid -3-5,7 mg/dl, serum alkaline phosphatase-98-279 UI/L, total serum calcium -8,6-10,2 mg/dl, ionised serum calcium -4.2-5,2 mg/dl, serum phosphorus-2,7-4,5 mg/dl, serum magnesium -1,6-2,6 mg/dl, urinalysis and urinary sediment, urinary protein level, regular values <140 mg/24h, microalbuminuria regular values <30 mg/24h.
- **Other biochemical samples**: glycaemia, regular values 74-115 mg/dl, blood iron -59-158 mg%, total serum proteins, 6,2-8 g/dl, serum potassium level 3,3-5,1 mmol/L, serum sodium level 136-145 mmol/L.

- **Microbiological samples:** urinalysis, culture of the uretheral secretion, culture of the vaginal secretion, PAP smear (test), Chlamydia trachomatis, Ureaplasma urealyticum, Mycoplasma hominis, HPV tests
- **Immunological samples:** AgHBs, AcHCV, CD4 level, regular values 330-1640 cel/mm3, CD4/CD8 1-3,7 ratio, TSH (thyroid stimulating hormone) regular values 0,25-4 UI/ml, FT4 (free thyroxine) 10,6-19,4 pmol/l, ATPO (TPO antibodies) regular values < 0.8 UI/ml, Antithyroglobuline antibodies regular values <115 UI/ml, intact parathyroid hormone (iPTH) regular values 15-65 pg/ml, 25 OH vitamin D, severe deficiency < 20 ng/ml, insufficient 21-29 ng/ml, optimal 30-55 ng/ml , RPR CARBON antigen (rapid plasma reagin), TPHA test (treponema pallidum hemagglutination test).
- **Molecular biology samples:** viral RNA HIV (Quantitative determination).

The value is deemed within the regular limit of the haematological samples, biochemical and immunological samples have been those set and used by the protocols of the Lab of the Clinical Hospital of Infectious Diseases of Constanta.

The diagnosis of HIV infection has been initially based on the serum tests for each reactive test taking a second sample, updating their determination through another performed method. The two screening tests were: the immuno enzymatic technique ELISA – Enzyme Linked Immunosorbent Assay, and respectively the immunochemical method – Chemiluminescence. The subsequent confirmation has been performed by determining the HIV viremia, and in some cases through the Western-Blot test.

Determining the cell mediated immunity and HIV-RNA viremia levels

In order to determine the immunological and virological status of the HIV seropositive patients, the number of CD4 lyphocytes/mm³ has been determined, the CD4/CD8 ratio, as well as the HIV RNA viremia levels (log10 copies/mL).

The cell mediated immunity tests I've been performed by lymphocyte immunophenotype in peripheral blood, using a monoclone mix of antibodies differentiating the following Lymphocyte subpopulations (sets the percentage of each lymophocyte subpopulation of the total lymphocytes): lymphcytes T (CD3+), lymphcytes T CD4 (CD3+ CD4+) and lymphcytes TCD8+ (CD3+CD8+) using the flow cytometry method with the help of Facs Count Manufactured by Becton Dickinson and Aquios.

Determination of the viral charge tests (no of RNA copies HIV/ml) have been done with two systems of automated real-time PCR : until 2016 with COBAS AmpliPrep/COBAS TaqMan, Roche and m2000, Abbott with a detection limit < 48 copies/ml, and from 2016, GeneXpert Manufactured by

Cepheid, with a detection limit 40 copies/ml. The tests performed using both methods and of the association of analysing apparatus and reactors are international accredited for the in vitro diagnosis (brand IVD), fulfilling the quality requirements for the parameters related to the sensitivity, specificity, precision, linearity of the quantification, including subtypes and correlation between their methods:

1. Minimum analytical sensitivity threshold 40 copies/mL

2. Specificity of 100% absence of cross-reactivity with the main microorganisms (viruses, bacteria, fungi) from the human pathology

3. Linearity of quantification interval from the minimum of 1,60 until 7,00 log copies/mL (40-10,000,000 copies/mL)

4. Covering the whole viral subtype spectrum HIV-1 identified at present (subtypes A-H of the group M and group O), including the subtype circulating in Romania and the recombined variants in the global circulation)

5. A good correlation between the two methods, with did the differences

Subtyping of HIV-1 has been performed using the algorithm REGA HIV- 1 and 2 automated subtyping tool version 2.0 Available at http://www.bioafrica.net/regagenotype/html/subtypinghiv.html. The REGA Basis represents a virtual analysis and management environment for the data of the infectious diseases field, which is publicly available and allows for the storage and analysis of the viral genetical sequences.

Screening- of the viral hepatitis (C and B) – has been done by using immunoenzymatic kits, type ELISA in order to identify the antibody anti viral hepatitis C, and respectively for the antigen HBs. In patients with positive Ag HBs the screening for the Delta hepatic virus has been performed, by testing the antibodies anti-VHD. **The screening of the thyroid hormones, the hormone iPTH, 25 OH vitamin D levels** has been done by the immunochemical assay with detection by electrochemical luminescence (ECLIA). **The haematological samples, complete blood count (CBC)** have been done using the optical impedance assay, using the apparatus MINDRAY_BC5380. **The Serum biochemistry samples** have been done by spectrophotometric assay using the apparatus KONELAB PRIME 301. The complete urinary examination (sediment and urinalysis) has been done by microscopic/photometric assay and through their reflectometric assay, respectively. **The urinary proteins in 24 hour urine collection** have been done by the turbidimetric assay**. Microalbuminuria in 24 hour urine collection** has been done by the immuno – turbidimetric assay. **Serum potassium and serum natrium level** have been done by

potentiometric method (ISE) **RPR CARBON and TPHA** tests have been done by latexagglutination and hemagglutination assay.

Imagering investigations

Abdominal and pelvic ultrasound and thyroid and parathyroid ultrasound exams have been done by Gianina Gabriela Șotilă, MdPh using the ultrasound apparatus General Electric LOGIQF8. The ultrasound assessment has been done at least two times a year, during all of the visits of the patients in the study group, during the follow-up period 2014-2019. For the patients that needed **URO-CT** or **Angio-CT,** these investigations have been performed using the apparatus GE Revolution EVO. **The parathyroid scintigraphy obtained through the radiopharmaceutica assay** have been done with Tcm^{99}-MIBI and Tcm^{99} SESTAMIBI in a dose of 740 MBq using a „Washout"' protocol. Early acquisition in 10 minutes from the injection, static, and SPECT-CT in late acquisition in two hours and 30 minutes from injection. **The kidney scintigraphy** have been done with Tc-DTPA. The **DEXA test has been done using the bone densitometer** Hologic DXA Wi System and Stratos whole body.

Statistic assessment

For the statistical activity involved in the study, the following types of variables have been defined: **categorical variables** used to determine: the gender, ethnicity, environment, known source of infection with HIV; Categorical variables involving a dichotomous variables, yes / no answer (1/0) used for a series of risk factors: smoking, viral HIV-1-F subtype F, TB securely or active, viral hepatitis B or C or D, diabetes mellitus, arterial hypertension, cardiovascular diseases, neoplasia, dyslipidaemia, metabolic syndrome, liver steatosis, renal urinary lithiasis, nephrocalcinosis, urinary infections, genital infection, Fanconi syndrome, renal or bladder malformation, thyroid impairment, DEXA testing, exposure to antiretroviral treatment, renal scintigraphy, parathyroid scintigraphy, URO-CT, Angio-CT, proteinuria, haematuria, glycosuria, granular and hyalin cylinders, crystals and **Continuous or discrete numerical variables used to determine**: age, the year when the HIV infection was found, classification of the CDC upon the diagnosis of HIV infection, the value of CD4 upon discovery ofthe HIV infection, the value of CD4 upon entering and exiting the study, the nadir CD4, the ratio CD4/CD8, value of RNA HIV level log 10, eGFR CKD-EPI, eGFR MDRD, serum creatinine, serum urea, serum uric acid, HgB, HCT, MCV, HEM, CHEM, blood iron, total serum proteins, serum tryglycerides, HDL and LDL cholesterol, total cholesterol, serum tryglycerides, glycaemia, total serum calcium and ionised serum calcium, serum phosphorus, serum alkaline phosphatase, 25 OH vitamin D, iPTH hormone.

The experimental data have been treated using the statistical operation program IBM SPSS Statistics 23. Procedures used: Descriptive statistics (to characterise the discrete and continuous variables in the database), charts, parametric statistic tests (Test t for independent variables, Test t for depending variables, One-way ANOVA test), non-parametrical tests - dealing with categorical variables (χ^2 association test, out of two category variables), χ^2 Test comparing two ratios , non-parametrical statistic tests for ordinal data or for dealing with a number of variables when the regularity condition is not satisfied (Mann-Whitney U, Kruskal-Wallis), concordance analysis, Kaplan-Meier analysis along with the test Lok-Rank used for the comparison of various survival curves, the Cox proporitional-hazards regression analysis. For all tests, the significance level has been of $\alpha = 0,05$.

Finally conclusions of the research

1. The HIV infection continues to represent a fundamental and profound public health probleme, with notable effects on the medical, economic and social system. Chronic kidney disease is a major comorbidity of HIV infection and a real probleme in evolution, healthcare and medical assistance of seropositive patients.
2. In Romania, chronic kidney disease associated of HIV infection is being clearly under diagnosed in PLWH, do to the fact that the definition and classification of the CKD is unknown, but also due to the fact that the calculus means for the glomerular filtration rate are in their turn underused.
3. The delay in diagnosing the kidney dysfunction and the loss of the therapeutical timeframe during which we could intervene, determins frequent therapeutical failures, given the kidney failure degree, medication overdose and secondary kidney toxicity, leading to an increase of morbidity and mortality of the HIV infected patients, by adding a new and important risk factor. The consequences of this medical misdiagnosed are devastating: the patients referral late to their nephrologist when does CKD is already advanced come on with important alterations of the glomerular filtration rate, frequently under 60 ml/min/1,73m², when severe complication of the CKD occur (anemia, occurance or aggravation of the arterial hypertension, metabolic acidosis, mineral and bone disease, etc), when the means of slowing down the disease progression has become inefficient.
4. The study shows that HIV infection is associated with a very high risk of developing chronic kidney disease, 20,6% respectively 14,7% of patients developing after 5 years of monitoring chronic kidney disease stage 1 (CKD-EPI-MDRD), 67,6% respectively 70,6% stage 2, 10,3% respectively 13,2% stage 3 and 1,5% stage 4, worrying percentages considering that the mean age of the group was 35 years and the subjects did not have major comorbidites. Progresul bolii cronice de rinichi a fost semnificativ în lotul nostru, pe parcursul celor 5 ani de urmărire, valoarea medie a eGFR calculată prin formula CKD-EPI, a scăzut cu 32,85 ml/min/1,73m², adică cu 6,57 ml/min/1,73m² pe an respectiv cu 36,15 ml/min/1,73m², adică cu 7,23 ml/min/1,73m² pe an, respectiv prin formula MDRD.
5. The progress of chronic kidney disease was significant in our group, during the 5 years of follow-up, the mean value of eGFR calculated by the CKD-EPI formula decreased by 32,85 ml/min/1,73m², meaning by 6,57 ml/min/1,73m² per year, respectively with 36,15 ml/min/1,73m², meaning by 7,23 ml/min/1,73m² per year, respectively by the MDRD formula.

6. The increased risk for the development of chronic kidney disease in patients with HIV infection is determined by the synergist action of several factors, being identified in our study by Cox regression analysis, both predictors specific to chronic kidney disease, independent of viral infection, such as diabetes mellitus, arterial hypertension, dyslipidemia, kidney stones, renal nephrocalcinosis as well as factors specific to HIV infection and antiretroviral therapy such as exposure to atazanavir or exposure to atazanavir-tenofovir or HIV associated coinfections such as hepatitis C or B. Renal lithiasis and renal nephrocalcinosis analyzed in the study were not evaluated as predictors for chronic kidney disease in HIV infection in any other national or international study.
7. These independent predictors will underlie the design of risk scores for the development and progression of chronic kidney disease that may be applicable in clinical practice to assess the nephrotoxicity of antiretroviral therapy or to calculate the risk that naive patient with HIV infection may have to develop chronic kidney disease in the next 5 years, respectively 10 years.
8. The study showed that the estimated risk of HIV infection patients to progress from stage 1 to stage 2 of chronic kidney disease was not influenced by the patients' sex, race, background, smoking or nonsmoking status. Instead, it was influenced by the mode of transmission of HIV infection and the age of the patients.
9. The research simultaneously used two methods for calculating the glomerular filtration rate, both eGFR CKD-EPI and eGFR-MDRD, both equations proving to be moderately concordant.. In these conditions, we recommend for the screening and monitoring of chronic kidney disease in HIV-positive patients the concomitant use of these two equations in all newly discovered patients with HIV infection and befor initiating antiretroviral therapy, the methods being non-invasive, reproducibl, cost- effective, affordable, quickly diagnosing the chronic kidney disease, the consequence being the reduction of costs due to prolonged hospitalizations and expensive medical care in this category of patients.
10. The calculation of eGFR by CKD-EPI has the advantage of a lower number of errors and higher accuracy in the seropositive population with normal or increased glomerular filtration rate, the MDRD equation underestimating eGFR in patients with normal renal function, being useful when the rate of glomerular filtration rate is below 60 ml/min/1,73m2. The use of these methods must be integrated in the field of modern international research, in order to be validated in patients with HIV infection, as they are not currently used in clinical practice in Romania.

11. The research led to a deeper understanding of the type of kidney injury and the connections between kidney disfunction-HIV infection- antiretroviral therapy. In the group of the 68 patients analyzed and monitored for a period of five years, a significant percentage of patients have been diagnosed with renal stones and renal nephrocaslcinosis. Most of these patients were exposed to the class of protease inhibitors. Given the increased risk of renourinary lithiasis and renal nephrocalcinosis, the protease inhibitors are to be avoided in the HIV-infected patient with CKD. We promote the use of new molecules, highly efficient for the HIV virus, with a good tolerability and renal safety.
12. In our study we identified a HIV patient profile with HIV infection and nephrolithiasis and/or nephrocalcinosis. Nephrolithiasis and nephrocalcinosis have been more frequent in the age range 20-29 years, predominantly in males, for those with the viral subtype F, for those with an advanced stage of the disease , with a level below 200 cells /mm³ of the CD4 lymphocytes upon discovery of the HIV infection and with the level of CD4 nadir under 200 cell/mm³ and with long exposure to protease inhibitors or INRT- Tenofovir exposure.
13. Due to the hight percentage of patients who developed kidney stones and renal nephrocalcinosis, we consider it necessary and appropriate to perform renal ultrasound at the initial evaluation of all patients with HIV infection, then repeated at 3, 6, 12 month, very useful imaging screening method, cost-effective, accessible and non-invasive.
14. The study showed a particular form of nephrolithiasis, called nephrocalcinosis, a common cause of recurrence of urinary tract infections.Due to the high percentage (44,1%), in selected cases, it is useful to complete investigations with screeni ng of serum and urinary ionogram, screening for metabolic disorders (urinary oxalate, urinary citrate, urinary calcium in urine/24 hours, 25 OH vitamine D hormone, iPTH hormone, osteodensitometry test), advanced medical imaging techniques such as URO-CT, if renal function allows the use of the contrst agent.
15. Tubular proteinuria, renal glycosuria with normoglycaemia, granular cylinders, significant leukocyturia, hipouricemia, hypophosphatemia, hypocalcemia may be considered early markers of incomplete proximal tubulopathysecondary to tenofovir fumarate use. The Fanconi syndrome or complete proximal tubulopathy secondary to tenofovir administration has been seen on 2,9% of the patients and glycosuria with normoglycaemia on 5,9% of the patients. In the seropositive patient on antiretroviral therapy, urine examination, especially microscopic examination of urinary sediment in light microscopy, can provide valuable information about the occurrence of tubulointerstitial

nephropathy or acute tubular necrosis secondary to antiretroviral medication, especially in patients on tenofovir fumarate, being considered a true „in vitro renal biopsy''.

16. The malformations, abnormalities and dysmorphic aspects of the renourinary apparatus must be taken into account in positive patient having a secondary kidney impairment, due to the fact that they are frequent, come in different forms, are misdiagnosed and allow for multiple complications, with different severity degrees, like the urinary tract infections, kidney urinary lithiasis, or progression of the CKD.
17. In the study group, the pyelocaliceal duplicity has been the most frequent congenital abnormality of the upper urinary ducts (35,3%), followed by congenital a small kidney (2,9%), horseshoe kidney (1,5%), pyelouretheral junction syndrome (1,5%) and congenital bladder diverticuli (1,5%). The nephrectomy performed for various kidney dysmorphic situations, such as multicystic kidney dysplasia and pyelouretheral junction syndrome IV-th degree complicated with pyonephrosis ocuured in 2,9% cases.
18. The incidence of sexually transmitted diseases such as syphilis in HIV-positive patients remains high, despite the advances made by modern medicine in diagnosing, treating and curing most of these diseases, representing an important substrate for the presence and recurrence of urinary tract infections.
19. Evaluation of renal dysfunction in patients with HIV-1-F viral subtype revealed that 33 subjects (88,84%) and 35 subjects (92,11%) of the total of 38 patients with HIV-1-F viral subtype, respectively progressed to chronic kidney disease stage 2 (eGFR CKD-EPI-MDRD) in 24 months.
20. Comparing the mean values of the glomerular filtration rate at the entrance and exit of the study, in patients with HIV-1-F viral subtype, there is a decrease of eGFR by 6,86 ml/min/1,73m² per year respectively 7,11ml/min/1,73m² per year (CKD-EPI, MDRD)
21. Staging of chronic kidney disease after 5 years of monitoring, showed that 21,1% of subjects with HIV-1-F viral subtype were in stage 1 of CKD, 71,1% were in stage 2 of CKD, 5,3% were in stage 3 of CKD and 2,6% were in stage 4 of CKD, alarming percentages because 76,3% (29 patients) belonged to the age range 20-29 years old.

Personal contributions

The research conducted and revealed in the our research reveals a new and multidisciplinary approach for screening, diagnosis and monitoring of the early renal dysfunction in HIV infection, the data obtained during the study complete the current gaps in knowing information about the type, degree and severity of renal injury in HIV infection, offering the possibility to improve prognostic and increasing the quality of life of the seropositive patients. It is the first study in Romania of such magnitude.

The contribution of the research results from identifying that non-invasive diagnosis means, such as calculating the glomerular filtration rate by two formulas CKD-EPI and MDRD, able to detect and assess the renal dysfunction from HIV infection, on an early stage, knowing the asymptomatic character of the CKD, up until the advanced stages, as well as identifying the cost of the therapy for replacing the renal function and associated comorbidities common both to the HIV infection and to the CKD.

In the future, we will need large scale national studies to demonstrate the validation of these formulas in HIV –positive patients in Romania. Any research study investigating the early discovery of the renal injury will facilitate the appearance and development of efficient prevention strategies for slowing down the kidney dysfunction and the degrading of the kidney function, which will lead to a considerable improvement of the clinical state and of the quality of life of the people infected with HIV.

The existance and design of National Registers for the monitoring of chronic kidney disease in patients with HIV infection would also be useful. The need to establish a medical specialization such as InfectoNephrology or HIV-Nephrology is a priority in the near future of modern patient-centered medicine. This would, in faact, allow a deepending, accuracy and detailed information on the latest discoveries and trends in the field of HIV-Nephrology. In this context, it becomes imperative to include a nephrologist in the multidisciplinary team that cares for the patient with HIV infection with multiple comorbidities.

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