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First investigation on microcrystalline pathologies of kidney allografts through cellular scale physicochemical techniques

Premières investigations physicochimiques à l'échelle cellulaire des microcalcifications du greffon rénal

Arnaud Lionet [a, *], Marie Haeck [a], Antoine Garstka [a], Viviane Gnemmi [b], Dominique Bazin [c, d], Emmanuel Letavernier [e, f, g], Jean-Philippe Haymann [e, f, g], Christian Noel [a], Michel Daudon [e, f, g]

[a] *Service de Néphrologie et Transplantation rénale, Hôpital Huriez, Lille, France*
[b] *Institut de Pathologie, CHRU-Lille, Lille, France*
[c] *Laboratoire de Physique des Solides, Université Paris Sud, Orsay, France*
[d] *CNRS-LCMCP-UPMC, Collège de France, Paris, France*
[e] *Service des Explorations Fonctionnelles Multidisciplinaires AP-HP, Hôpital Tenon, Paris, France*
[f] *Université Pierre-et-Marie-Curie, Paris-6, Paris, France*
[g] *INSERM, UMR S 1155, Paris, France*

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ABSTRACT

Tubulo-interstitial microcalcifications in renal transplant are described with a wide difference of incidence (4–78%) according to time and goal of biopsies. Currently, staining procedures are used to deduce the composition of crystals and speculate about their aetiologies. Here we test the contribution of infrared microspectroscopy (IR-MS) in understanding kidney transplant crystal deposits. First, microcalcifications observed in 118 allograft biopsies are studied by IR-MS. The Fourier transform infrared signal shows that a major proportion (92%) of calcium phosphate crystals is in the pure or mixed form. Next, we compare 50 patients with calcifications to 100 without calcifications and show persistent hyperparathyroidism and tubular cell vacuolization as circumstances of crystal deposition. Finally, the graduation level of calcification by IR-MS appears to be correlated with the graft outcome. Graft survival seems to be worse in case of high microcalcification detection by IR-MS. These preliminary data suggest IR-MS as a great tool for clinicians to diagnose, characterize, and quantify microcalcifications in kidney allografts.

RÉSUMÉ

L'incidence des cristaux tubulo-interstitiels au sein des greffons rénaux est très variable (4 à 78%) selon le motif et le délai de réalisation de la biopsie. La nature des cristaux est déduite de leurs aspects en microscopie optique selon les différents marquages utilisés pour ensuite orienter le clinicien sur leurs étiologies. Nous testons ici l'apport de la microspectroscopie infrarouge (MS-IR) dans l'étude des dépôts cristallins du greffon rénal. Tout d'abord nous étudions en MS-IR la nature des microcalcifications de 118 biopsies de

* Corresponding author. Service de Néphrologie et Transplantation rénale hôpital Claude Huriez CHRU-lille, Lille, France.
E-mail address: arnaud.lionet@chru-lille.fr (A. Lionet).

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greffon. Il s'agit majoritairement (92%) de cristaux purs ou mixtes phosphocalciques. Ensuite, la comparaison de 50 patients avec calcifications à 100 témoins permet d'identifier l'hyperparathyroidie et les lésions de microvacualisations tubulaires comme étant associées aux dépôts cristallins. Enfin, l'abondance de ces dépôts est quantifiée par MS-IR. Elle semble corrélée au pronostic de la greffe, avec une survie moins bonne du greffon en cas de dépôts abondants. Ces données préliminaires suggèrent que la MS-IR est un outil performant pour le diagnostic, la caractérisation et la quantification des microcalcifications du greffon rénal.

1. Introduction

Composition, aetiology and consequences of microcalcifications in kidney allografts are unclear. Widely different incidences of crystal deposits have been described, ranging from 4 to 78% [1–8]. First descriptions were in favour of calcium oxalate deposits as a consequence of delayed graft function and serious tubular necrosis [1,9]. More recently, studies have pointed out the responsibility of mineral disorder present after renal transplantation in the constitution of calcium phosphate deposits [4,10]. The impact of such deposits in renal outcome is conflicting. Inconsistencies in studies are in part explained by different biopsy protocols and histological evaluation. Staining procedures give different information about crystals in renal transplant biopsies. Pathologists take advantage of Von Kossa staining to highlight the calcium deposits [11]. Note that a recent investigation has underlined some drastic limitations regarding such staining procedures [12]. The Pizzolato staining is generally used for the demonstration of calcium oxalate crystals in paraffin [13]. Polarized light permits distinction between calcium phosphate and calcium oxalate crystals [6]. But none of these techniques are able to distinguish the chemical nature in different phases as spectrometry can. Infrared microspectroscopy (IR-MS) is now available to study accurately crystals in human tissue [14–16]. This technique permits to screen the presence of microcalcifications in a sample biopsy and to determine their chemical compounds [17]. The present study aims to illustrate the contribution of IR-MS in (a) the physicochemical characterisation of crystal deposits, (b) the understanding of the circumstances of such depositions, and (c) the outcomes of allografts with microcalcifications.

2. Materials and methods

2.1. Study population

We performed a retrospective single-centre observational study: 118 biopsy samples were retrospectively retrieved from a key-word search of the computed database of the Department of Pathology of the University Hospital of Lille. The selection terms consisted of "calcification", "crystal" and "graft kidney". The selection criteria included availability of the paraffin-embedded kidney allograft biopsy tissue. These 118 biopsies were performed in 72 allograft recipients. One hundred patients without crystals in their biopsy were randomised as controls. All patients consented to participate in the protocol. Patients were treated with double immunosuppressant of calcineurin inhibitor and mycophenolate mofetil, and prednisolone was added in case of sensitisation. This study was approved by our local ethics committee. Informed consent was obtained for biopsy and for the use of clinical data and secondary use of histological materials for research.

2.2. Biopsy analysis

2.2.1. Histopathological analysis

The study population consisted of protocol biopsies performed 3 months after transplantation and biopsies for renal dysfunction. Two tissue cylinders were obtained by percutaneous renal transplant biopsy using a biopsy gun with a 16-gauge needle. One cylinder was fixed in buffered formalin and embedded in paraffin for routine light microscopic examination. Slices of biopsy samples in paraffin were routinely stained by Massons' trichrome, HES, PAS, and Jones methods. The other tissue cylinder was frozen for immunofluorescence microscopy analysis. All biopsies were evaluated according to the updated BANFF classification. In case of visualisation of crystalloid deposits during microscopy examination, biopsies were submitted to IR-MS analysis.

2.2.2. IR-MS analysis

IR-MS was performed on an IN10MX microscope (Thermo Scientific, ZA Courtaboeuf, Les Ulis, France). Tissue samples were deposited on Low-e microscope slides (MirrIR, Kevley Technologies, Tienta Sciences, Indianapolis) and then chemically treated with xylene to remove paraffin in order to improve crystal detection. All spectra were collected in ultrafast mode using a 50 $\mu m \times 50\ \mu m$ aperture. The spectra were collected in the 4000–800 cm^{-1} mid-IR range at a resolution of 16 cm^{-1} with one spectrum per pixel. Data analysis of IR spectra and chemical images was performed using OMNIC software. Infrared spectroscopy was also carried out on a PerkinElmer Spotlight 400 FTIR microspectrometer fitted with a liquid nitrogen cooled, 16-element linear array mercury cadmium telluride (MCT) detector. All IR spectra were collected in the mid-infrared from 4000 cm^{-1} to 650 cm^{-1} using 16 cm^{-1} spectra resolution and 64 accumulations for each collection by the array. The different compounds were identified by comparing them to reference spectra [18]. Results were classified in pure or mixed chemical deposits, with an intensity range of spatial distribution in the sample tissue from 1 to 4.