Laboratory diagnosis of chronic kidney disease in adults: an overview of hospitals inserted in the Portuguese National Health System

Diagnóstico laboratorial de doença renal crônica em adultos: visão geral dos hospitais inseridos no Sistema Nacional de Saúde de Portugal

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ABSTRACT

Introduction: The laboratory diagnosis of chronic kidney disease (CKD) is a simple and cost-effective procedure that allows the detection of early stages of the disease, which is essential to avoid kidney damage and a life-threatening event. It consists of measuring serum creatinine concentration, urinary albumin concentration and calculating the estimated glomerular filtration rate (eGFR). In 2012, the guidelines for laboratory evaluation of the CKD were published by the Kidney Disease: Improving Global Outcomes (KDIGO). Objectives: This study aimed to evaluate whether the laboratories in hospitals of the Portuguese National Health System follow these guidelines and provide a correct diagnosis of CKD. Material and method: A questionnaire composed of 32 questions was sent to the Clinical Pathology Services of all hospitals inserted in the System. Results: All 49 labs responded that measure serum creatinine, 18 reported measuring eGFR. Ten reported measuring eGFR only if specifically ordered. Forty-four measure total protein and albumin in the urine, three only protein, one albumin alone, and one measure none of them. The type of samples, methods, reagents, equipment, expression units of results and reference intervals varied. Conclusion: There is great variability among laboratories in relation to the methodology of measuring serum creatinine, albumin and total protein in the urine. There are wide variations in the release of results. Most laboratories do not follow the guidelines recommended by the KDIGO 2012. This work indicates that there is a need to develop education and alignment processes in the laboratory diagnosis of CKD in the laboratories installed in hospitals inserted in the Portuguese National Health System.

Key words: chronic kidney failure; glomerular filtration rate; benchmarking.

INTRODUCTION

Chronic kidney disease (CKD) is a major public health problem worldwide, reaching about 10% of the Portuguese population(1). Early diagnosis of CKD is essential to prevent severe and life-threatening kidney damage. This can be achieved by performing simple and inexpensive laboratory tests, along with proper management and interpretation of the results.

In 2012, guidelines for the evaluation of CKD have been released by the Kidney Disease: Improving Global Outcomes (KDIGO)(2), updating the National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF KDOQI) guidelines released in 2002(3). These guidelines aim to improve the assessment, management and treatment of patients with CKD. Laboratory assessment of the estimated glomerular filtration rate (eGFR), a measure of kidney function and albumin in the urine, which measures kidney damage, are the key elements for the laboratory diagnosis of this disease and recommendations on how to properly measure these parameters are presented in this document.
OBJECTIVE

The aim of this study was to access the state-of-the-art of the laboratory diagnosis of CKD in Portugal, aiming to its standardization according to the KDIGO guidelines recommendations.

A national survey was performed in order to evaluate the laboratory diagnosis of this disease in Portuguese public hospitals. Data were obtained from all laboratories.

MATERIAL AND METHOD

Survey development

A survey composed of 32 questions (22 closed and 10 open questions) was developed covering essential topics for the diagnosis of CKD (“serum creatinine”, “eGFR”, and “urine total protein and urinary albumin”). A topic on “Quality control” was also included in order to access the liability of laboratory assays. “Education” and “Confidentiality” topics were included in order to understand the position of laboratories regarding further information on this issue and to preserve their privacy rights, although the results were never intended to be presented individually or to reveal the identity of the hospital.

Health care institutions involved

The survey was sent by e-mail to the responsible(s) of the Clinical Pathology Service of all 49 hospitals inserted in the Portuguese National Health System. Data were collected between August and December, 2016.

RESULTS

A total of 49 (100%) hospitals answered the survey.

Serum creatinine

All hospital laboratories in this study measure serum creatinine. Compensated Jaffe method traceable to isotope dilution mass spectrometry (IDMS) reference was the most common method, preferred by twenty-eight (58.1%) laboratories. The analytical methods used and the respective prevalence are shown in Figure 1. Thirty-four (69.4%) laboratories measure creatinine using standardized methods (compensated Jaffe and compensated enzymatic method, both IDMS calibrated). There is a high variability in the manufacturers of the reagents used in serum creatinine measurement, as well as the equipment used.

Forty (81.6%) laboratories report creatinine values in mg/dl, one in µmol/l and eight in both units. One laboratory presents creatinine values in nmol/l and mg/dl.

Fourteen (28.6%) laboratories report creatinine values with one decimal place, thirty three (67.4%) with two decimal places, one (2.0%) with no decimal places and one (2.0%) with both, none and two decimal places, according to the units in which creatinine value is reported.

The reference interval used by the laboratories is highly variable, with thirty-two (65.3%) laboratories that distinguish reference values between men and women.

eGFR

Eighteen (36.7%) laboratories report the eGFR value, eight (16.3%) of which along with serum creatinine measurements and ten (20.4%) only when specifically ordered by the physician (see Figure 2). Thirty-one (63.3) do not report eGFR and ten of them do not consider reporting it in the near future.

The chronic kidney disease-epidemiology collaboration (CKD-EPI) and the Modification of Diet in Renal Disease-version 4 (MDRD-4) for standardized creatinine are the most prevalent equations used (8/18 and 6/18 respectively). Other equations and respective prevalence are shown in Figure 3.

Four laboratories report an eGFR value “higher than” when values are above 60 ml/min/1.73 m², two report it when values are above 90 ml/min/1.73 m², and twelve always report the exact number.

Concerning the parameter race, the equations mentioned by two laboratories do not include it (Cockcroft-Gault and Hoek’s-cystatin C formulas) and sixteen use race-dependent equations.
to measure urine protein is highly variable (Figure 4), with some laboratories accepting more than one type of sample. However, the most prevalent is the 24-hour urine sample (41/47), followed by first morning and random urine samples. The measuring methods are predominantly turbidimetry (23/47) and colorimetry (20/47) (Figure 5). The units in which urinary protein are reported are also highly variable, with maximum values reaching 300 mg/g creatinine or equivalent.

With regards to albumin, the preferred sample is predominantly the 24-hour urine sample (38/45) (Figure 6), and the methods more prevalent to its measurement are turbidimetry (29/45) and nephelometry (9/45) (Figure 7). The units in which urine albumin are reported are highly variable and some laboratories report it in more than one unit, with the majority of the laboratories reporting it in mg/24 hour (37/45), mg/l and mg/g creatinine (14/45 and 15/45, respectively). The cut-off values for albumin in the urine are also highly variable, with maximum values reaching 80 mg/g creatinine or equivalent.

There is a high variability in the manufacturers of the reagents used in the measurement of urine protein and albumin, as well as the equipment used, with no markedly preferred brand.

Thirty laboratories report the use of albumin to creatinine ratio (ACR). It was not clear by the questionnaire if they report this ratio along with all measurements of albumin and creatinine in the urine or only when specifically ordered by the physician.

Quality control

From the 49 laboratories studied, 32 use internal and external quality control (IQC and EQC, respectively) programs for all the parameters measured. The 16 remaining laboratories have one or

Urine proteins and albumin

From the 49 laboratories involved in this survey, 44 measure both total proteins and albumin in the urine, three measure only total protein, one measures only albumin, and one none of those. The type of sample
Confidentiality

All laboratories allowed the divulgation of the survey’s data, taking into account that this study does not specify the hospitals in any of the questions.

DISCUSSION

Note: all recommendations mentioned are based on the KDIGO 2012 Clinical Practice Guideline for the evaluation and management of CKD(2).

Serum creatinine

Although it is not the ideal filtration marker, serum creatinine is the most widely used and recommended by the KDIGO guidelines as an initial assessment of CKD. Standardized assays calibrated with the IDMS method must be performed in order to minimize intra-laboratory variations and enzyme assays should be preferred since they allow a reduction in bias and interferences. All laboratories in this study measure serum creatinine and 69.4% use standardized assays IDMS calibrated. From these, six use enzyme assays.

The reagents and equipment used to measure serum creatinine are highly variable among laboratories. However, since methods are standardized, this probably is not the origin of the disagreements between the diagnoses.

All laboratories report creatinine values in units recommended by the KDIGO in mg/dl and/or μmol/l. Recommendations are the following: when units are reported as mg/dl, creatinine values should display two decimal places, and when reported as μmol/l, creatinine values should be rounded to the nearest whole number. This is fulfilled by 55.1% of the laboratories. Most of them presenting creatinine values in both mg/dl and µmol/l (7/8) presented only one option for the number of decimal places used and, therefore, were not included as following the recommendations. Concerning the reference interval for normal creatinine, there is no recommendation on this value in the KDIGO guidelines, but there is the need for its standardization among laboratories, along with standardization of reagents, methods and equipment if possible.

eGFR

The eGFR value should be reported along with serum creatinine values, for initial CKD assessment. Eighteen laboratories measure eGFR, eight of which along with serum

Education

From the 49 laboratories participating in this study, 47 have stated that they want to receive further information for the implementation of national guidelines concerning the parameters covered by the survey.
creatinine measurements and ten only when specifically ordered by the physician. The most prevalent equations are CKD-EPI (8/18) and MDRD-4 for standardized creatinine (6/18). Thirty-one laboratories do not report eGFR and from these, ten do not consider to report it in the near future. These decisions will difficult a national standardization for laboratory diagnosis of CKD.

The eGFR value should be calculated from the standardized creatinine measurements. Thirteen laboratories reporting eGFR measure creatinine by calibrated and standardized methods. The only equations suitable to measure eGFR from standardized creatinine values are CKD-EPI and MDRD-4 for standardized creatinine. Eleven laboratories reporting eGFR measure creatinine by calibrated and standardized methods use CKD-EPI (7/11) and MDRD-4 (4/11) for standardized creatinine equations and, therefore, they are following the recommendations. Three laboratories using CKD-EPI and MDRD-4 equations for standardized creatinine measure creatinine levels by not compensated and not calibrated methods.

MDRD-4 equation produces inaccurated and biased results when eGFR is higher than 60 ml/min/1.73 m². These results are greatly improved when using the CKD-EPI equation. Therefore, CKD-EPI equation is the one recommended by the KDIGO guidelines, except in situations where it has been shown that another equation is more suitable for the population in study. However, MDRD-4 equation for standardized creatinine is also accepted.

Using CKD-EPI equation, eGFR values should be reported as exact numbers (recommendation followed by 5/8 laboratories) and levels below 60 ml/min/1.73 m² should be reported as “decreased”. When using MDRD-4 equation, a value higher than 60 ml/min/1.73 m² should be reported as > 60 ml/min/1.73 m² (recommendation followed by 2/6 laboratories).

The parameter “race” is part of both CKD-EPI and MDRD-4 for standardized creatinine equations. It should be reported as “Black” or “White”. Seven laboratories using these equations do not include the parameter “race” in it. In terms of easeness, it is easier for the laboratory not to include the parameter race, attention that needs to be taken by the physician that reads the result.

Variations of eGFR values in some special situations, such as admitted patients and pregnant women, although not having any recommendation by the KDIGO, are highlighted by some laboratories (8/18). Thirteen laboratories release eGFR values for admitted patients, although it is known that, in this situation, this value should not to be valued.

The only laboratory that reports eGFR for children (1/18) uses the original Schwartz equation instead of the recommended Updated “Bedside” Schwartz formula.

**Urine protein and albumin**

Albumin is the main protein found in the urine of CKD patients. It can also be standardized and allows an improvement in the sensitivity, specificity, quality and consistency tests, facts for which it is recommended for total proteins in initial assessment of CKD. However, since there are some situations in which total proteins may be necessary to measure, and measuring albumin is considerably more expensive than total proteins, therefore, questions regarding total urine proteins measurement were also included in this survey.

Forty-four laboratories in this study measure both albumin and total proteins in the urine. Three measure only protein, one measures only albumin and one none of those.

The first morning void urine is the preferred sample for both proteins and albumin measurement in the urine, it is well correlated with 24-hour protein, has low intra-individual variability, is ordered for orthostatic proteinuria diagnosis and is less susceptible to errors during collection. Random urine samples are also accepted as initial tests for albuminuria and proteinuria. Nevertheless, most laboratories recommend a 24-hour urine sample for total protein (87.2%) and for albumin (84.4%) assessment. A first morning sample is preferred by ten laboratories when measuring urine protein, and by twelve laboratories when measuring urine albumin. Concerning the measurement methods, turbidimetry and colorimetry are preferred to measure total protein (48.9% and 42.6%, respectively), and turbidimetry and nephelometry to measure albumin (64.4% and 20.0%, respectively). Immunoassay should be preferred when measuring albumin, due to their precision at low concentrations and production of quantitative results in the clinically relevant range. This is accomplished by 84.4% of the laboratories.

There is a high variability in the manufacturers of the reagents used in the measurement of urine protein and albumin, as well as the equipment used, with no markedly preferred brand. There are no recommendations on these issues in the KDIGO 2012 guidelines, but standardization would be advantageous for the same reasons mentioned on the analysis of the parameter “serum creatinine”.

Concerning reporting units for both protein and albumin, these should be reported in relation to urine creatinine (mg/mmol
creatinine or mg/g creatinine), instead of a total concentration alone, allowing correction for variations in urinary concentration due to hydration state. Ten laboratories that measure protein in the urine report values in mg/g creatinine. Thirteen laboratories that measure albumin in the urine report values only in mg/g and two in both, mg/g and mg/mmol creatinine.

Concerning the cut off values for proteins, a maximum value of 150 mg/g creatinine or equivalent should be considered (followed by 6/10 laboratories) and for albumin, a maximum of 30 mg/g creatinine or equivalent should be considered (followed by 13/15 laboratories). Reporting units and cut-off values should be aligned among laboratories, in order to facilitate reading and evaluation of the results by the physicians.

Thirty laboratories that report albumin in the urine also report the albumin to creatinine ratio (ACR). However, it is not clear if this ratio is calculated along with albumin measurements or only when ordered. Ideally, it should be reported preferably to albumin concentration alone, as recommended by the KDIGO guidelines. A protein to creatinine ratio (PCR) should also be preferred to total protein concentration alone, but this matter was not address in our survey.

Quality control

The use of Quality Control Programs, when measuring laboratory tests is of extreme importance to ensure reliability and accuracy of the results obtained. IQC programs allow the internal monitoring of a parameter, and thus evaluating the accuracy of the result. EQC allows the comparison of a specific parameter to a group of laboratories and confirms the results from IQC, evaluating the accuracy of results. Both are important and complementary and evaluate the quality of the results generated by the laboratory. Thirty-two laboratories in this study use IQC and EQC programs when measuring the parameters “Serum creatinine”, “Urine proteins” and “Urine albumin”. Two laboratories do not perform one of the controls and fourteen do not evaluate two or more controls. It is important that all laboratories carry out IQC and EQC programs for all parameters and, therefore, further education on this issue is required.

FINAL CONSIDERATIONS

To the best of our knowledge, this is the first study on laboratory diagnosis of CKD in adults in Portugal. The results obtained from this study show a high variability among the hospitals inserted in the Portuguese National Health System with respect to laboratory diagnosis of CKD in adults. The KDIGO guidelines have not been implemented in most of the hospitals. Only one laboratory follows the recommended and preferred guidelines. Nine laboratories follow recommended guidelines. Standardization of laboratory diagnosis of CKD is a necessary and urgent step, which will allow:

- a more correct and effective diagnosis of CKD;
- the same individual diagnosis, regardless the hospital in which the laboratory tests are performed.

This study evidences a question of alignment and standardization that, in the limit, can compromise the diagnostic efficiency, delaying the identification of patients at risk of CKD. The authors encourage similar studies in other communities and a commitment to reduce heterogeneity.

QUESTIONNAIRE

Survey “Laboratory diagnosis of CKD-Portugal” (Chart).

ACKNOWLEDGMENTS

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CONFLICTS OF INTEREST

We have no conflict of interest to declare.
<table>
<thead>
<tr>
<th>Questions</th>
<th>Answers</th>
</tr>
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<tbody>
<tr>
<td>1) Does your laboratory measure serum creatinine?</td>
<td>• Yes (go to the next question)</td>
</tr>
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<td></td>
<td>• No (go to question no. 17)</td>
</tr>
<tr>
<td>2) Please indicate the method used to measure serum creatinine values</td>
<td>• Not compensated Jaffe</td>
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<td>(more than one can be selected).</td>
<td>• Compensated Jaffe, IDMS calibrated</td>
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<tr>
<td></td>
<td>• Compensated enzymatic method, calibration to IDMS</td>
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<td></td>
<td>• Dry chemistry</td>
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<td></td>
<td>• Other(s) (please specify)</td>
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<tr>
<td>3) Please specify the reagent’s manufacturer(s) to measure serum</td>
<td></td>
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<tr>
<td>creatinine.</td>
<td></td>
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<td>4) Please specify the type of instrument(s) in which serum creatinine is</td>
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<tr>
<td>measured.</td>
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<td>5) In which unities of values the serum creatinine for adults</td>
<td>• mg/dl</td>
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<td>(&gt; 18 years old) are reported?</td>
<td>• µmol/l</td>
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<td></td>
<td>• both</td>
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<td>6) How many decimal places are presented in the creatinine results?</td>
<td>• One</td>
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<td></td>
<td>• Two</td>
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<td></td>
<td>• More (please specify)</td>
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<tr>
<td>7) Which is the reference interval used by your laboratory for serum</td>
<td></td>
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<tr>
<td>creatinine in adults?</td>
<td></td>
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<tr>
<td>8) Does your laboratory report the eGFR along with the determinants</td>
<td>• Yes, along with serum creatinine measurement (go to question no. 11)</td>
</tr>
<tr>
<td>of serum creatinine?</td>
<td>• Yes, whenever ordered by the physician (go to question no. 11)</td>
</tr>
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<td></td>
<td>• No (go to question no. 10 and afterwards to question no. 17)</td>
</tr>
<tr>
<td>9) Does your laboratory consider reporting the eGFR?</td>
<td>• Yes</td>
</tr>
<tr>
<td></td>
<td>• No</td>
</tr>
<tr>
<td>10) Which equations/formulas does your laboratory use to determine the</td>
<td>• MDRD-4 for non-standardized creatinine</td>
</tr>
<tr>
<td>eGFR?</td>
<td>• MDRD-4 for standardized creatinine</td>
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<td></td>
<td>• MDRD-6</td>
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<td></td>
<td>• CKD-EPI</td>
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<td></td>
<td>• Cockroft-Gault</td>
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<td></td>
<td>• Other (please specify)</td>
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<tr>
<td>11) At what value does your laboratory point to an eGFR value higher than</td>
<td>• 60 ml/min/1.73 m²</td>
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<tr>
<td>(&gt;?)</td>
<td>• 90 ml/min/1.73 m²</td>
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<td></td>
<td>• Never. We always report the exact value</td>
</tr>
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<td></td>
<td>• Other (please specify)</td>
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<tr>
<td>12) How does your laboratory report the results concerning the parameter</td>
<td>• Two results, “Black” and “White”</td>
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<tr>
<td>“race”?</td>
<td>• Race evaluated during the appointment</td>
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<td></td>
<td>• Patient is asked to report its race</td>
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<td></td>
<td>• “Race” parameter is not included</td>
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<td></td>
<td>• Other (please specify)</td>
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<tr>
<td>13) In the eGFR result, there are included observations about possible</td>
<td>• Nutritional status</td>
</tr>
<tr>
<td>variations of this value with:</td>
<td>• Pregnancy</td>
</tr>
<tr>
<td></td>
<td>• Admitted patients</td>
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<tr>
<td></td>
<td>• We don’t include observations about possible variations of eGFR</td>
</tr>
<tr>
<td></td>
<td>• Other(s) (please specify)</td>
</tr>
<tr>
<td>14) Special situations: admitted patients.</td>
<td>• We report eGFR for admitted patients</td>
</tr>
<tr>
<td></td>
<td>• We don’t report eGFR for admitted patients</td>
</tr>
<tr>
<td>15) Which equations/formulas does your laboratory use to determine the</td>
<td>• Schwartz equation (original)</td>
</tr>
<tr>
<td>eGFR in children?</td>
<td>• Schwartz equation (modified)</td>
</tr>
<tr>
<td></td>
<td>• Other (please specify)</td>
</tr>
<tr>
<td></td>
<td>• We don’t calculate eGFR for children</td>
</tr>
<tr>
<td>Urine protein and albumin</td>
<td>• Total protein in urine</td>
</tr>
<tr>
<td></td>
<td>• Total albumin in urine</td>
</tr>
<tr>
<td>16) Do you perform the following tests in your laboratory?</td>
<td>• None of those (go to question no. 31)</td>
</tr>
</tbody>
</table>
17) Which is the recommended sample to measure total protein in urine?
- 24-hour urine sample
- Random urine sample
- First morning urine
- Not specified
- Other (please specify)

18) Which method does your laboratory use to measure total protein in urine?
- Nephelometry
- Turbidimetry
- Colorimetry
- Dry chemistry
- Other (please specify)

19) Please specify the reagent’s manufacturer(s) to measure total protein in urine.

20) Please specify the type of instrument(s) in which total protein in urine is measured.
- mg/mmol creatinine
- mg/g creatinine
- g/24 h
- g/l

21) How the values of total protein in urine are reported?
(More than one option can be selected)

22) Which is the reference interval for total protein in urine in your laboratory?
- 24-hour urine sample
- Random urine sample
- First morning urine
- Not specified
- Other (please specify)

23) Which is the recommended sample to measure albumin in urine?
- 24-hour urine sample
- Random urine sample
- First morning urine
- Not specified
- Other (please specify)

24) Which method does your laboratory use to measure albumin in urine?
- Nephelometry
- Turbidimetry
- Colorimetry
- Dry chemistry
- Other (please specify)

25) Please specify the reagent’s manufacturer(s) to measure albumin in urine.

26) Please specify the type of instrument(s) in which albumin in urine is measured.
- mg/mmol creatinine
- mg/g creatinine
- µg/min
- mg/24 h
- µg/ml
- mg/l

27) How the values of albumin in urine are reported?
(More than one option can be selected)

28) Which is the reference interval for albumin in urine in your laboratory?

29) Does your laboratory report the ACR?
- Yes
- No

Quality control

30) Concerning the parameters “serum creatinine”, “total protein in urine” and “albumin in urine”, which quality control program do you use?
- “Serum creatinine” – IQC
- “Serum creatinine” – EQC
- “Urine protein” – IQC
- “Urine protein” – EQC
- “Urine albumin” – IQC
- “Urine albumin” – EQC

Education

31) Are you interested in obtaining information for the implementation of clinical guidelines to use in laboratory daily tasks, concerning the parameters referred in this survey?
- Yes
- No

Confidentiality

32) Do you have any restriction to the divulgation of any of these data?
- Yes, I don’t want any of these data to be released
- Yes, I don’t want the information of some of the questions to be released (please specify)

IDMS: isotope dilution mass spectrometry; eGFR: estimated glomerular filtration rate; MDRD-4: Modification of Diet in Renal Disease version 4; MDRD-6: Modification of Diet in Renal Disease version 6; CKD-EPI: chronic kidney disease-epidemiology collaboration; ACR: albumin/creatinine ratio; IQC: internal quality control; EQC: external quality control.
RESUMO

Introdução: O diagnóstico laboratorial de doença renal crónica (DRC) é simples e econômico e permite a detecção de estágios iniciais da doença, o que é essencial para evitar danos renais e risco de morte. Consiste em medir a concentração de creatinina sérica e albumina urinária e calcular a taxa de filtração glomerular (eTFG). Em 2012, as diretrizes para avaliação laboratorial da DRC foram divulgadas pela Kidney Disease: Improving Global Outcomes (KDIGO). Objetivos: Os objetivos deste estudo são avaliar se os laboratórios em hospitais do Sistema Nacional de Saúde Português seguem essas diretrizes e se fornecem diagnóstico correto de DRC. MATERIAL E METODO: Um questionário com 32 perguntas foi enviado aos serviços de patologia clínica de todos os hospitais inseridos no sistema. Resultados: Todos os 49 laboratórios responderam que medem creatinina e 18, eTFG. Dez disseram que medem a eTFG apenas se especificamente solicitado. Quarenta e quatro medem proteínas totais e albumina urinária; três, apenas proteínas; um, somente albumina; e um não mede nenhuma delas. Tipo de amostras, métodos, reagentes, equipamentos, unidades de expressão dos resultados e intervalos de referência variaram. Conclusão: Existe grande variabilidade entre laboratórios em relação às metodologias de medida da creatinina sérica, albumina e proteínas totais na urina. Há grandes variações quanto à liberação dos resultados. A maioria dos laboratórios não segue as diretrizes recomendadas pela KDIGO 2012. Este trabalho indica que existe necessidade de serem desenvolvidos processos de educação e harmonização no diagnóstico laboratorial de DRC nos laboratórios instalados em hospitais inseridos no Sistema Nacional de Saúde Português.

Unitermos: falência renal crónica; taxa de filtração glomerular; benchmarking.

REFERENCES


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