CONCLUSIONS

1. S'ha establert que el període de màxima estabilitat en el posttrasplantament renal per estudiar variabilitat biològica comprèn un interval de 8 determinacions, que comença entre 10-15 dies i dos mesos després del trasplantament segons el pacient, i es manté durant una mitjana de tres mesos.

2. El protocol assistencial de seguiment dels pacients trasplantats renals és un bon model per estudiar variabilitat biològica.

3. L'obtenció de mostres en intervals de temps idèntics no és, en el posttrasplantament renal, imprescindible per estudiar variabilitat biològica.

4. S´ha estimat la variabilitat biològica intraindividual i interindividual en el posttrasplantament renal.

5. Els constituents estudiats més apropiats pel seguiment del posttrasplantament renal són creatinina, urea i urats.

6. S'ha comprovat que existeix independència entre les concentracions de creatinina i urats en el període d'estabilitat clínica dels pacients trasplantats renals.

7. S'ha determinat el valor de referència d'un canvi (VRC) per creatinina i urats combinats a diferents intervals de probabilitat.

8. L'aplicació dels VRC de creatinina i urats combinats incrementa el poder predictiu de canvis significatius en l'evolució dels pacients trasplantats renals.

9. S'ha elaborat un model d'enquesta dirigida als nefròlegs per optimitzar el valor calculat del VRC, tenint en compte l'actuació clínica en el seguiment del pacient.

10. Pel que fa a la sensibilitat, l'especificitat, els valors predictius i els quocients de probabilitat, els VRC_s de creatinina de 18.1% i d' urats de 19.8% (interval de probabilitat del 85%) presenten la millor combinació, per a detectar rebuig o complicacions renals.

11. Les opinions dels clínics especialistes corroboren la utilització dels VRC_s creatinina i urats combinats (18.1% i 19.8%, respectivament) pel que fa a la sensibilitat i l'especificitat obtingudes.

12. Es verifica la utilitat clínica del model VRC combinat (creatinina i urats). La predicció de rebuig i de complicacions es constata en pacients que es troben en situació idèntica als utilitzats en el model descrit en aquesta tesi.

CONSEQÜÈNCIA PRÀCTICA

El laboratori clínic on ha estat realitzada aquesta tesi estudiarà incorporar en el sistema informàtic un algoritme de càlcul dels VRC_s que s'apliqui a tots els pacients posttrasplantats renals i als resultats de creatinina i urats.
Així mateix els resultats de creatinina i urats que han superat els valors de referència d'un canvi assignats, s'assenyalaran amb un símbol que ho identifiqui.

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ANNEX - QÜESTIONARI

1. Qüestions generals

· ***Estat actual del trasplantament renal:***

- De quins mitjans es disposa pel diagnòstic a temps d'un rebuig agut ? (pensar en els pacients que han sofert un trasplantament renal i que presenten un curt període d'estabilitat clínica.)
- Es fa sempre el diagnòstic postrebuig agut, confirmatori: Biòpsia renal?.

· ***Estadística actual del trasplantament renal:***

- Evolució del posttrasplantament renal. Quina és la distribució aproximada en percentatges de la seva evolució?.

· ***Importància de la "no detecció precoç d'un rebuig agut".***

- Quines són les conseqüències pel pacient de la "no detecció precoç d'un rebuig agut?.
- Quines són les conseqüències econòmiques, tractament, etc.?.

· ***Proves bioquímiques:***

- Quina consideres que és la fiabilitat de les proves bioquímiques que s'utilitzen per al seguiment del pacient posttrasplantat renal?.
- Quines proves bioquímiques utilitzes per al diagnòstic del rebuig agut?. Citar proves.
- Quina utilitat tenen els resultats dels urats en la monitorització del pacient posttrasplantat renal?.
- Quins són els percentatges entre dues determinacions seriades que utilitzes per a detectar canvis significatius en l'evolució clínica del pacient posttrasplantat renal?. Especifica en quines proves bioquímiques.

- Els percentatges crítics entre determinacions seriades que apliques en el seguiment del posttrasplantament renal és fruit de l'experiència i pràctica clíniques o de la consulta bibliogràfica?. Si és bibliogràfica, quina és la bibliografia de referència?.

· ***Conseqüències d'un resultat positiu fals:***

- Quines són les conseqüències d'un diagnòstic de "rebuig agut" *Fals* ?. Pacient que diagnostiques i pautes tractament com rebuig agut i després comproves que no és.

1. Pel pacient.
2. Econòmic.
3. Valorar si les conseqüències que hagués estat cert i no s'hagués actuat a temps serien molt pitjors.

· ***Conseqüències d'un resultat negatiu fals:***

- Quines són les conseqüències de no haver diagnosticat un "rebuig agut" a temps?. Pacient que creus que segueix estable i que després comproves que pateix un rebuig agut.

1. Pel pacient.
2. Econòmic.

· ***Protocol de seguiment del pacient posttrasplantat renal:***

Sempre que el pacient posttrasplantat renal segueix una evolució normal, Quins són els motius més comuns que poden modificar el protocol de seguiment del posttrasplantament renal? (referit a les proves bioquímiques). És molt crític el fet que no el segueixi?. El segueixen la gran majoria?.

2. Qüestions basades en casos clínics:

1. Al Sr. Rj-18 de 27 anys d'edat se li ha practicat un trasplantament renal fa 4 setmanes amb bona evolució. El resultat de creatinina en el seu control habitual és de 179 µmol/L. Al cap de 15 dies acudeix de nou per al seu control segons el protocol de seguiment del pacient posttrasplantat. L'estat del pacient segueix normal i la concentració de l'immunosupressor també.
   A partir de quina concentració de creatinina (increment respecte al resultat anterior, mínim), prendries alguna acció respecte a la seva evolució?.
   Resposta:

- El mateix cas si el resultat de creatinina és de 91 µmol/L?
  Resposta:

2. Al Sr. Rj-18 de 27 anys d'edat se li ha practicat un trasplantament renal fa 4 setmanes amb bona evolució. El resultat d'urats en el seu control habitual és de 414 µmol/L. Al cap de 15 dies acudeix de nou per al seu control segons el protocol de seguiment del pacient posttrasplantat. L'estat del pacient segueix normal i la concentració de l'immunosupressor també.
   A partir de quina concentració d'urats (increment respecte al resultat anterior, mínim), prendries alguna acció respecte a la seva evolució?.
   Resposta:

- El mateix cas sent el resultat d' urats de 249 µmol/L?
  Resposta:

3. Al Sr. Rj-18 de 27 anys d'edat se li ha practicat un trasplantament renal fa 4 setmanes amb bona evolució. El resultat de creatinina és de 179 µmol/L i el d'urats és de 414 µmol/L en el seu control habitual. Al cap de 15 dies acudeix de nou per al seu control segons el protocol de seguiment del pacient posttrasplantat. L'estat del pacient segueix normal i la concentració de l'immunosupressor també.

   A partir de quina concentració de creatinina i urats (increment respecte al resultat anterior, mínim), prendries alguna acció respecte a la seva evolució?.

   Resposta:

- El mateix cas si el resultat de creatinina és de 91 µmol/L i el d'urats de 249 µmol/L?

  Resposta:

4. El Sr. Rj-16 de 26 anys d' edat , trasplantat renal des de fa 2 setmanes, presenta un valor de creatinina de 276 µmol/L. Al cap de 3 dies acudeix de nou a control segons el protocol de seguiment establert. El resultat de creatinina és de 363 µmol/L. El resultat d' urats no varia pràcticament del resultat anterior, l'evolució del pacient és normal i la concentració de l'immunosupressor també.

   Quina probabilitat creus que té de sofrir un rebuig agut en els propers 5-6 dies?.

   Resposta:

   a) 0 - 50%
   b) 50 - 80%
   c) 80 – 100 %

5. El Sr. Rj-16 de 26 anys d' edat , trasplantat renal des de fa 2 setmanes, presenta un valor de creatinina de 276 µmol/L i d'urats de 376 µmol/L. Tres dies més tard acudeix de nou a control segons el protocol de seguiment establert. El resultat de creatinina és de 363 µmol/L i el d'urats de 509 µmol/L. L' evolució del pacient és normal i la concentració de l'immunosupressor també. ***Quina probabilitat creus que té de sofrir un rebuig agut en els propers 5-6 dies?.***

   Resposta:

   a) 0 - 50%
   b) 50 - 80%
   c) 80 – 100 %

6. El Sr. Rj-16 de 26 anys d' edat , trasplantat renal des de fa 2 setmanes, presenta un valor d'urats de 376 µmol/L. Tres dies més tard acudeix de nou a control segons el protocol de seguiment establert. El resultat d'urats és de 509 µmol/L . El resultat de creatinina no varia pràcticament del resultat anterior, l'evolució del pacient és normal i la concentració de l'immunosupressor també. ***Quina probabilitat creus que té de sofrir un rebuig agut en els propers 5-6 dies?.***

   Resposta:

   a) 0 - 50%
   b) 50 - 80%
   c) 80 – 100 %

7. El Sr Rj-13 de 40 anys d'edat, trasplantat renal des de fa 10 dies, presenta un valor de creatinina de 83 µmol/L. Tres dies més tard acudeix al seu control habitual sense símptomes . El resultat de creatinina és de 99 µmol/L, la concentració d'urats no varia pràcticament i la de l'immunosupressor està dins dels nivells terapèutics. ***Quina probabilitat creus que té de sofrir un rebuig agut en els pròxims 10-12 dies?.***

   Resposta:

   a) 0 - 50%
   b) 50 - 80%
   c) 80 – 100 %

8. El Sr Rj-13 de 40 anys d'edat, trasplantat renal des de fa 10 dies, presenta un valor de creatinina de 83 µmol/L i d'urats de 249 µmol/L. Tres dies més tard acudeix al seu control habitual sense símptomes . El resultat de creatinina és de 99 µmol/L, la concentració d'urats és de 302 µmol/L, la de l'immunosupressor està dins dels nivells terapèutics. ***Quina probabilitat creus que té de sofrir un rebuig agut en els pròxims 10-12 dies?.***

   Resposta:

   a) 0 - 50%
   b) 50 - 80%
   c) 80 – 100 %

9. El Sr Rj-13 de 40 anys d'edat, trasplantat renal des de fa 10 dies, presenta un valor d'urats de 249 µmol/L. Tres dies més tard acudeix al seu control habitual sense símptomes . El resultat d'urats és de 302 µmol/L, la concentració de creatinina no varia pràcticament, la de l'immunosupressor està dins dels nivells terapèutics. ***Quina probabilitat creus que té de sofrir un rebuig agut en els pròxims 10-12 dies?.***

   Resposta:

   a) 0 - 50%
   b) 50 - 80%
   c) 80 – 100 %

10. El Sr. Rj-2 de 20 anys d'edat ha sofert un trasplantament renal fa 4 setmanes i ve a la consulta per a la seva monitorització i control. El valor de creatinina és de 126 µmol/L. Tres dies més tard la concentració de creatinina és de 173 µmol/L, l'estat general és normal i la concentració d'urats i d'immunosupressors també.

    Numera segons el teu criteri i experiència el que faries per ordre de prioritats a partir de 1 (1 = la primera acció, la més important), fins el número que calgui segons el número d'accions que creguis convenient portar a terme.

- Ecografia
- Determinació de creatinina: nova sol·licitud de creatinina al dia següent.
- Examen clínic del pacient.
- Determinació analítica de l'immunosupressor (Csa, o FK).
- Determinació d' un altre constituent bioquímic. Si és així, quin?.
- Canvi de tractament. Quin?.
- Hospitalització del pacient per al seu estudi.
- No faria res, esperaria la propera visita de control que li toqui segons el protocol.
- Una altra actuació diferent. Especifica-la.
- Biòpsia renal.

11. El Sr. Rj-2 de 20 anys d'edat ha sofert un trasplantament renal fa 4 setmanes i ve a la consulta per a la seva monitorització i control. El valor de creatinina és de 126 µmol/L i el d'urats és de 285 µmol/L. Tres dies més tard la concentració de creatinina és de 173 µmol/L i la concentració d'urats és de 454 µmol/L. L'estat general és normal i la concentració d'immunosupressors també.

    Numera segons el teu criteri i experiència el que faries per ordre de prioritats a partir de 1 (1 = la primera acció, la més important), fins el número que calgui segons el número d'accions que creguis convenient portar a terme.

- Ecografia
- Determinació de creatinina: nova sol·licitud de creatinina al dia següent.
- Examen clínic del pacient.
- Determinació analítica de l'immunosupressor (Csa, o FK).
- Determinació d' un altre constituent bioquímic. Si és així, quin?.
- Canvi de tractament. Quin?.
- Hospitalització del pacient per al seu estudi.
- No faria res, esperaria la propera visita de control que li toqui segons el protocol.
- Una altra actuació diferent. Especifica-la.
- Biòpsia renal.

12. El Sr. Rj-2 de 20 anys d'edat ha sofert un trasplantament renal fa 4 setmanes i ve a la consulta per a la seva monitorització i control. El valor d'urats és de 285 µmol/L. Tres dies més tard la concentració d'urats és de 454 µmol/L, l'estat general és normal i la concentració d'immunosupressors també.

    Numera segons el teu criteri i experiència el que faries per ordre de prioritats a partir de 1 (1 = la primera acció, la més important), fins el número que calgui segons el número d'accions que creguis convenient portar a terme.

- Ecografia
- Determinació de creatinina: nova sol·licitud de creatinina al dia següent.
- Examen clínic del pacient.
- Determinació analítica de l'immunosupressor (Csa, o FK).
- Determinació d' un altre constituent bioquímic. Si és així, quin?.
- Canvi de tractament. Quin?.
- Hospitalització del pacient per al seu estudi.
- No faria res, esperaria la propera visita de control que li toqui segons el protocol.
- Una altra actuació diferent. Especifica-la.
- Biòpsia renal.

13. A la Sra. Rj-8 de 50 anys d' edat se li va fer un trasplantament renal fa 4 mesos. Ve a realitzar-se el control habitual segons el protocol. El resultat de creatinina és de 279 µmol/L. Quatre dies més tard el valor de creatinina és de 331 µmol/L, l'estat general és normal i la concentració d' urats i de l'immunosupressor també.

    Numera segons el teu criteri i experiència el que faries per ordre de prioritats a partir de 1 (1 = la primera acció, la més important), fins el número que calgui segons el número d'accions que creguis convenient portar a terme.

- Ecografia
- Determinació de creatinina: nova sol·licitud de creatinina al dia següent.
- Examen clínic del pacient.
- Determinació analítica de l'immunosupressor (Csa, o FK).
- Determinació d' un altre constituent bioquímic. Si és així, quin?.
- Canvi de tractament. Quin?.
- Hospitalització del pacient per al seu estudi.
- No faria res, esperaria la propera visita de control que li toqui segons el protocol.
- Una altra actuació diferent. Especifica-la.
- Biòpsia renal.

14. A la Sra. Rj-8 de 50 anys d' edat se li va practicar un trasplantament renal fa 4 mesos i ve a realitzar-se el control habitual segons el protocol. El resultat de creatinina és de 279 µmol/L i la concentració d'urats de 360 µmol/L. Quatre dies més tard el valor de creatinina és de 331 µmol/L i el d'urats de 469 µmol/L, l' estat general és normal i la concentració de l'immunosupressor també.

    Numera segons el teu criteri i experiència el que faries per ordre de prioritats a partir de 1 (1 = la primera acció, la més important), fins el número que calgui segons el número d'accions que creguis convenient portar a terme.

- Ecografia
- Determinació de creatinina: nova sol·licitud de creatinina al dia següent.
- Examen clínic del pacient.
- Determinació analítica de l'immunosupressor (Csa, o FK).
- Determinació d' un altre constituent bioquímic. Si és així, quin?.
- Canvi de tractament. Quin?.
- Hospitalització del pacient per al seu estudi.
- No faria res, esperaria la propera visita de control que li toqui segons el protocol.
- Una altra actuació diferent. Especifica-la.
- Biòpsia renal.

15. A la Sra. Rj-8 de 50 anys d' edat se li va practicar un trasplantament renal fa 4 mesos i ve a realitzar-se el control habitual segons el protocol. El resultat d'urats és de 360 µmol/L. Quatre dies més tard el valor d'urats és de 469 µmol/L, l' estat general és normal i la concentració de l'immunosupressor també.

    Numera segons el teu criteri i experiència el que faries per ordre de prioritats a partir de 1 (1 = la primera acció, la més important), fins el número que calgui segons el número d'accions que creguis convenient portar a terme.

- Ecografia
- Determinació de creatinina: nova sol·licitud de creatinina al dia següent.
- Examen clínic del pacient.
- Determinació analítica de l'immunosupressor (Csa, o FK).
- Determinació d' un altre constituent bioquímic. Si és així, quin?.
- Canvi de tractament. Quin?.
- Hospitalització del pacient per al seu estudi.
- No faria res, esperaria la propera visita de control que li toqui segons el protocol.
- Una altra actuació diferent. Especifica-la.
- Biòpsia renal.

ANNEX - PUBLICACIONS

A MODEL FOR ESTABLISHING BIOLOGICAL VARIATION IN NON HEALTHY SITUATIONS: RENAL POST-TRANSPLANTATION DATA

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SUMMARY

We managed analytic data from the routine monitoring protocol for kidney transplant recipients according to the fundamentals of biological variation to see if early indicators of rejection could be established. A group of 19 patients who had received a kidney graft were studied for a period of two years. Using six serum quantities that were expected to reflect instability/rejection, we delineated the period of relative stability after renal transplatation. Within- and between-subject biological variation, indices of individuality and critical differences between serial results were calculated for these constituents. The duration of period of maximum stability varied,averaging three months. The indices of individuality showed that creatinine, urate and urea are suitable for monitoring. The critical differences for all three constituents were around 28%. An interpretation of serial results from the combination of these three constituents could be a method for early detection of crises in renal post-transplantation patients.

Indexing terms: renal function, transplantation, monitorization, variation source of, index of individuality, critical difference.

Abbreviations: s^2_a analytical imprecision, s^2_{i+a} within subject plus analytical variation,

s^2_i ,s^2_gwithin and between subject variation,respectively, s^2_t total variation,

I.I. index of individuality , CD critical differences, ANOVA analysis of variance.

Renal transplantation has come to be accepted as standard treatment for patients suffering from terminal kidney failure. Since the physiological state of the renal transplant recipient is unstable, he is monitored with a well-defined protocol that is strictly followed by clinicians. Renal dysfunction is a common complication due to various problems, especially drug toxicity, and rejection is an ever-present danger (1). To detect episodes of rejection, clinicians use empirical criteria derived from experience that is mainly based on changes in creatinine levels. Post-transplantation monitorization, which includes frequent analysis of a number of constituents, generates large amounts of data. Using this information, it would be beneficial to find objective, early indicators that predict trends toward complications before the patient's condition is seriously affected, so that preventive actions can be taken.

Data from biological variation (BV), the normal fluctuation around the homeostatic setting point, has been used to evaluate the significance of changes in serial results (2). Therefore, it can provide clinicians with an indication of future patient status: a change between two consecutive observations higher than the established variation around the

homeostatic setting point, could signal the beginning of a complication. However, the components of biological variation, sensitive and specific enough to characterize a "certain state of health" from the start of crisis, can be investigated only when a stable situation that denotes equilibrium has been demonstrated in the specific pathology.

The aims of this work were: to delineate the stable period after renal transplantation for six serum quantities expected to reflect instability/rejection; and to calculate within- and between-subject biological variation, indices of individuality and critical differences between serial results and compare outcome with published data in healthy subjects, to determine whether BV data can predict crises in this population.

We studied nineteen patients (12 men and 7 women), 19 to 64 years old, with chronic renal insufficiency, who had received an orthotopic kidney graft. Permission for enrollment in the study was obtained from all patients, as required by the Helsinki II protocol.

Serum specimens were collected according to the usual hospital follow-up protocol designed by the nephrologists for these patients, as summarized:

First week post-transplantation: daily.

From the second to fourth week: twice a week.

From the first to the third month: weekly.

From the third to the sixth month: every 15 days.

From the sixth to the twelfth month: once a month.

From the first year on: every two or three months, indefinitely.

Stability in postransplanted patients is routinely verified through a combination of clinical, analytical and imaging parameters: clinical normality as indicated by symptomatology, physiological constants, diuresis, weight, physical examination, etc; analytical profile with particular vigilance on creatinine results (expected to differ less than 25% between two consecutive samplings) and doppler echography. The data from our 19 patients was studied for a period of two years post-transplantation, and during this time there was no evidence of crisis or rejection according to the nephrologists' protocol.

The conditions of specimen collection were standardized to minimize the effect of collection. Specimens were obtained into evacuated blood-collection tubes without anticoagulant. The specimens were allowed to clot at room temperature, centrifuged at 3000 x g for 15 min. We separated the serum, and quantities were analyzed. The study was conducted in real time throughout.

Six serum biochemistry quantities were determined: creatinine, urea, urate, sodium,

potassium and chloride.

Establishment of the homeostatic point.

The homeostatic point for each quantity was derived from the period of maximum stability.

a) *Determination of the beginning of the stable period.*

The analytic results from each quantity in each patient were represented graphically from the beginning of the post-transplantation period. Visual inspection of the graphs showed high results in the first determinations that decreased to a point after which results remained constant over time (from negative slope to horizontal lines) in some of the quantities. This point was considered to be the beginning of the stable period and was called "point zero". Point zero and the stable pattern were seen very clearly in the creatinine analyses and were confirmed in the urea graph, however in the remaining quantities a clear inflection point was not observed. Therefore in each patient, point zero for all the quantities studied was derived from the creatinine results.

b) *Determination of the end of the stable period.*

Results were normalized according to the start value (3): the ratio of each result from a patient with respect to his value at point zero was calculated.

The coefficient of variation (CV) of the ratios of the 19 patients (normalized CV) was calculated and the difference between the CV for each sampling day and the CV for point one (the CV at point zero is 0) were depicted on a graph, against the analytical variation of the method for each constituent. When the difference between the normalized CV was found to be higher than the analytical CV, stability was considered to have ended.

Biological variation calculations.

a) *Analytical imprecision* (s^2_a) was calculated through control materials, averaging the routine data for 12 months, and using the control concentration closest to the mean concentration values found in the 19 patients studied.

Before performing calculations with the patients' results, the Cochran test (4) was applied to exclude outlying values from the individual subjects, and the Reed test (5) to eliminate mean outlying values.

The ANOVA test (6) was used to estimate within-subject (s^2_i) plus analytical variation (s^2_{i+a}), expressed as the weighted mean of variances from the 19 patients.

b) *Within-subject (intraindividual) biological variation* (s^2_i) was calculated by a subtraction step with the two previous variables (s^2_{i+a}-s^2_a).

c) *Between-subject(group) biological variation* (s^2_g) was obtained by subtracting the within-subject plus analytical variation from the total variation (s^2_t) found using all data from all patients:

$$(s^2_g) = (s^2_t) - (s^2_{i+a})$$

d) *Indices of Individuality* (I.I.) were calculated using the formula:

$$I.I. = s^2_{i+a} / s^2_g$$

e) *Critical differences* (CD) between consecutive results were calculated after confirming with the Kolmogorov-Smirnov test (6) that the (s^2_{i+a}) data were normally distributed; subsequently, the one-tail formula (_=0.05) was used to detect only significant increases:

$$CD = 1.65 \times 2(s^2_{i+a})^{-} = 2.33\ (s_{i+a}) \quad (p<0.05)$$

To derive BV data, the stable period within the pathology has to first be defined: the more precisely the stable period is delineated, the more robust will be the indicators produced. The beginning of post-transplantation stability was determined by examining the

slopes of the quantities studied. We considered that a clear inflection (and subsequent maintenance) of the slope in more than one quantity simultaneously would signal the beginning of stability. For all 19 patients, creatinine and urea showed this pattern and were considered valid for our purposes.

The beginning of the stable period, was found between the first and second month post-transplantation, although the exact moment when it occurred was not necessarily the same in all patients. The fact that at this time the nephrologists' protocol reduces the required analyses to a frequency of one per week indicates that the empirically-based criteria also perceive stability at this time.

To minimize interindividual variation and to facilitate the detection of relative changes among patients, all results were normalized (3,7,8). Figure 1 shows the difference between the normalized CV at each sampling day and the CV at point one, in relation to the analytical CV (parallel line to the X axis) for creatinine. The period of stability was considered to conclude when the CV of the difference was higher than the analytical CV. This occurred within an interval of 8 determinations (that starts between one and two months after surgery), when follow-up protocol analyses are performed once a week, and is maintained for an average of three months, depending on the patient.

Table 1 shows the components of analytical and biological variation found in this study, expressed in terms of coefficients of variation. Data from healthy subjects obtained by averaging results from previous works (9,10), are also exhibited.

When studying the components of biological variation,the analytical component (CVa) is lower than half the within-subject component (CVi) for all constituents studied (except chloride and sodium), demostrating that the use of "real time" analytical data is appropiate for studying biological variation . Browning (11) recommended that when studing BV, the analytical component should be less than 20% of the total variance found. The analytical difficulties for chloride and sodium are similar than those found by other authors in similar studies (12)(13).

Biological variation data from healthy subjects have been compiled in two well-known articles (9,10). Few works have dealt with biological variation in pathological status. Fraser reported that the within-subject and between-subject variation for certain analytes in patients with renal dysfunction and cardiac infarction,are the same as in healthy subjects (13,14). Hölzel (15, 16) , however, shows discrepancies in specific pathological situations. Our data show that within-subject variation was higher in the kidney graft recipients than in the healthy population, being most evident in the creatinine, potassium, urate, and urea

results. We found no differences in the between-subject variation, except for potassium.

We studied biological variation to determine if analytical results from the routine monitoring protocol could be used as predictors of functional alteration in renal transplant recipients. To know which constituents are suitable as early indicators of negative evolution in pathological situations, the indices of individuality are determined. Creatinine, urate and urea, with I.I.s of approximately 0.6 are suitable for monitoring according to Fraser and Harris statements (2).

Harris proposed a formula derived from within-subject variation to interpret critical differences in serial results (17,18). We found that critical differences were around 28% in creatinine,urate and urea. This figure is very close to the 25% criteria used by the nephrologist in their protocol. Moreover non the 19 patients surpassed this differences over the study period indicating that no significant changes occurred.

The other constituents provided no information for the purpose of predicting funtional alteration. Thus, an interpretation of serial results from the combination of creatinine, urate and urea analyses could be a method for early detection of possible crises in post-transplantation patients.

In conclusion, this work describes a model for studying biological variation in a non

healthy state using available laboratory data. It shows a method for demonstrating stability within the pathology and for deriving the components of biological variation.

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Tittle to Figure 1:

Figure 1: End of the stable period determination.

Legend to Figure 1:

Each point represents the difference between normalized CV at each sampling day and CV at point one,in relation to the analytical CV. (CV_a=5,4% for creatinine).

ARE EQUALLY SPECIMENS NECESSARY TO ASSESS BIOLOGICAL VARIATION?. EVIDENCE FROM RENAL TRANSPLANT RECIPIENTS

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Key words: renal function, transplantation, monitoring, index of individuality

ABSTRACT

The established method for determining the components of biological variation (BV) requires equispaced time intervals between extractions. In a previous study, we determined BV in renal post transplantation patients, taking advantage of the samples obtained within their clinical treatment protocol (not necessarily equispaced). To confirm the validity of this practice, we sought to determine if the use of varying sampling intervals has an effect on the results obtained in such biological variation studies. The study included two phases: comparison of the results found with identical and non-identical sampling intervals and correlation between the within-subject BV and the length of the sampling interval. There were no differences in within-subject BV between the groups or correlations with sampling intervals for any of the constituents studied. We conclude that samples acquired within established clinical protocols for kidney transplant recipients can be used for estimating BV.

Abbreviations: BV= biological variation, CV_a = analytical coefficient of variation, CV_w = within-subject biological coefficient of variation, CV_{w+a}= within-subject biological plus analytical coefficient of variation, CV_b= between-subject biological coefficient of variation.

INTRODUCTION

Biological variation (BV), the physiological fluctuation in levels of body fluid constituents around the homeostatic setting point, has been widely used in laboratory medicine for many purposes, such as deriving analytical quality specifications, determining reference change values and assessing the utility of reference ranges. A large number of studies have been dedicated to estimating biological variation components (1-5), and the majority have used the model proposed by Fraser and Harris (6) for this purpose. This model was contemplated for use with healthy subjects, and was based on a strict protocol under controlled conditions. It has been found that some of these conditions, such as length of the study and number of subjects included do not have to be absolutely fixed, as was originally believed, because variations in these factors have not produced discrepancies in BV estimates (7-10). However, other conditions, including standardization of sample collection and well controlled analytical procedures, are absolutely necessary.

Since BV is concerned with fluctuations around a fixed point, when applying the study model to pathologies, it is important that the patients are in a relatively stable state to establish a new homeostatic setting point. In a previous study we demonstrated that there is a stable period after kidney transplantation, which made possible estimation of BV components in this group of patients (11). Although Fraser and Harris' model based on healthy subjects suggests that the time interval between sample collections should be equispaced, a small number

of BV studies have used samples taken at varying intervals, without placing attention on this factor (12-14).

When studying BV in non-healthy states, it is almost impossible to fulfil the requirement of equispaced sampling. Patients are usually submitted to exhaustive medical observations which, in many cases, imply serial laboratory tests with frequencies that depend on the evolution of the disease, the treatment prescribed, etc. In these cases, the researcher is not free to design the sampling interval. For ethical reasons, patients should not be submitted to more venepunctures than those requested by the monitoring clinician.

Our aim in this study was: 1. To elucidate if equispaced sampling interval is a determinant factor when estimating the components of biological variation in recipients of kidney allografts, and 2**.** To establish the components of biological variation in this non-healthy situation.

MATERIALS AND METHODS

Patients

The study included 41 subjects with chronic renal insufficiency (25 men and 16 women, 19-66 years old) who had received an orthotopic kidney graft. Informed consent was obtained from all participants, as required by the Helsinki II protocol. The period of maximum stability in this non-healthy situation had been determined in our previous study (11) and comprised an interval of eight samplings, beginning between 1 and 2 months after surgery, and maintained for an average of 3 months, depending on the patient.

Sample collection

Serum specimens were collected according to the standard hospital follow-up protocol designed by the nephrologists for renal transplant recipients, as summarized: a) first week post-transplantation, daily; b) from the second to fourth week, twice a week; c) from the first to the third month, weekly; d) from the third to the sixth month, every 2 weeks; e) from the sixth to the twelfth month, once a month, f) from the first year on, every 2 or 3 months, indefinitely. Specimens were analysed at the time of collection, not stored for batched analysis.

To establish two groups, one with regular and one with irregular sampling intervals, we studied the total of 41 patients individually and found 19 who had equispaced sampling for four specimens (group A). The second group (B) was formed by 19 patients (with four samples each) who had irregularly spaced sampling, selected randomly from the 22 remaining patients. We limited group B to 19 subjects to facilitate the comparison.

Six serum constituents were determined: creatinine, urea, urate, sodium, potassium and chloride, using the analytical procedures described previously (11).

Statistical analysis

Normal distribution of the results from constituents studied was confirmed in Groups A and B by means of the Kolmogorov - Smirnov test (15).

The homogeneity of the within-subject plus analytical variances between the two groups of patients was confirmed by use of the Snedecor tests (15).

We evaluated the relationships between the within-subject biological plus analytical coefficients of variation and the average time span between serial determinations in the total group of patients, by means of the Pearson coefficient correlation (15).

Biological variation components were calculated as we detailed in our previous study (11).

RESULTS AND DISCUSSION

1. Effect of sampling interval

Table 1 shows the mean and the within-subject biological plus analytical coefficients of variation (CV_{w+a}) obtained in the two groups of subjects. There were no statistically significant differences between the groups in within-subject plus analytical variances for any of the constituents studied, indicating that sampling periodicity has little influence on estimation of the components of biological variation. The within-subject plus analytical variance is expressed as coefficients of variation (CV_{w+a}).

To investigate whether length of sampling interval influenced BV results, we assessed the relationship between the within-subject biological plus analytical coefficient of variation (CV_{w+a}) and the time interval among the eight serial determinations obtained in the stable period, for each patient and each constituent. The within-subject biological plus analytical coefficient of variation showed no significant increase with longer sampling intervals in any of the constituents studied.

Table 2 shows the Pearson correlation coefficient between CV_{w+a} and average sampling intervals for the 41 patients studied, and the values of the CV_{w+a} and the sampling interval corresponding to the patients with the highest and the lowest CV_{w+a}. The patient with the highest CV_{w+a} was not the one whose

samples were obtained at longer time intervals, and similarly the patient with the lowest variability was not the one with shortest sampling intervals. This was confirmed for all the constituents in the study.

The comparison of results between Groups A and B, and the lack of correlation between within-subject biological plus analytical variation estimates and periodicity of sampling, demonstrated that samples do not need to be obtained at identical time intervals to estimate the components of biological variation.

2. Components of biological variation

The total group of 41 kidney recipients was studied to determine the components of biological variation in this non-healthy state, after verifying the hypothesis that results are not different when samples are obtained at varying or at constant time intervals. The number of stable patients available for study had increased since our earlier study (11) and we believed that the conclusions derived would be more representative if we re-calculated the components of biological variation with a larger number of patients over a longer study period (some 3 years). Table 3 shows the analytical and biological components of variation found. There were no notable differences in within-subject biological variation, with respect to the earlier study. Regarding between-subject biological variation, creatinine and potassium values were higher in the second study, probably because of the larger population of patients

CONCLUSIONS

Our results show that in the clinical situation described, the study of biological variation did not require sampling at identical time intervals. This finding opens the possibility for similar studies carried out in real time using the analytic determinations that are routinely practiced on the patients, without the need to perform unnecessary samplings or to modify the follow-up protocol established according to the state of health involved.

We believe that this model for studying biological variation in kidney post-transplanted patients can be applied to study biological variation in other non-healthy states that are under a strict follow-up protocol, after the period of stability has been established.

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We thank Per Hyltoft Petersen for his invaluable help in improving the orientation of this study.

Table 1. Mean and within–subject biological plus analytical coefficients of variation in patients with equispaced (group A) and variable (group B) sampling intervals

Constituent	Mean			CV_{w+a} %	
	Units	Group A	Group B	Group A	Group B
Creatinine	µmol/L	148	128	9.2	11.7
Urea	mmol/L	11.7	9.9	12.1	13.8
Urate	µmol/L	415	368	8.6	12.0
Sodium	mmol/L	141	140	1.6	1.6
Potassium	mmol/L	4.46	4.59	6.5	6.8
Chloride	mmol/L	108.6	109.3	3.0	3.0

Table 2. Relationships between CV_{w+a} and sampling intervals

ANALYTE	Correlation Coefficient	Highest CV_{w+a} (%)	Averaged Sampling Intervals (days)	Lowest CV_{w+a} (%)	Averaged Sampling Intervals (days)
Creatinine	0.0639	23.9	22	4.2	34
Urea	0.1483	32.3	25	5.1	34
Urate	-0.0549	24.3	14	3.7	24
Sodium	0.0288	3.1	14	0.6	34
Potassium	0.0607	13.6	14	4.0	14
Chloride	0.1202	4.6	14	1.1	14

Table 3. Analytical and biological coefficients of variation obtained in the group of 41 kidney post-transplanted patients

Constituent	CV_a %	CV_{w+a} %	CV_w %	CV_b %
Creatinine	4.8	12.3	11.5	23.7
Urea	4.3	15.5	15.3	25.1
Urate	3.4	13.5	13.2	20.2
Sodium	1.5	1.8	1.1	1.1
Potassium	2.0	7.3	7.1	7.3
Chloride	2.3	3.0	1.8	1.5

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REFERENCE CHANGE VALUE CONCEPT COMBINING TWO DELTA VALUES TO PREDICT CRISES IN RENAL POST-TRANSPLANTATION

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***Abbreviations**:* RCV, reference change value; BV, biological variation; CV_{w+a}, within-subject biological plus analytical coefficient of variation; z_p, factor for a certain probability; TN, true-negative test values; TP, true-positive test values; FN, false-negative test values; FP, false-positive test values.

The concept of “reference change value” (RCV) was developed by Harris and Yasaka (1,2) to identify significant changes in the state of patients when monitoring their pathology. This concept can be applied to the laboratory data routinely acquired for renal post-transplantation patients to detect potential crises before clinical indications are manifested. The RCV takes into account the within-subject biological variation (BV) as well as the analytical variation, when considering serial laboratory results. The distinction between pathologic change and laboratory “noise” can be improved to provide better information about the patient’s status, if the RCV is calculated when (3-7):

- The clinical situation is well defined and managed through a strict protocol.
- The reference group studied is homogeneous regarding the disease and treatment and is within a demonstrated period of stability.
- The event to predict is the same for all patients.
- The analytical procedure used is well-controlled.

In previous works using data from kidney transplantation patients (6,8) we noted that the quantities most suitable for detecting significant changes during post transplantation follow up are serum creatinine, urate and urea. Now, we think that the predictive power of these analytes would be greatly increased if more than one simultaneously evidenced significant changes, before the clinical manifestations of reduced kidney function became apparent, while the fluctuations in their concentrations were independent and not influenced by each other.

For this reason, the purpose of this work is to provide an objective analytical indicator (using these analytes) to detect potential subclinical crises in renal transplant recipients based on the reference change value concept. To make the model relevant for use in daily practice, we attempted to include the nephrologists' criteria in the final values proposed, by determining the factors they consider crucial when monitoring their transplant patients(9)(10). The underlying philosophy of this effort is to extract the most information possible from routine laboratory data and offer the clinician an improved tool for patient care.

To test whether the concentrations of creatinine, urea, and urate were independent, pairs of data from the three quantities in the same individuals and the same samples during the stable period obtained in the previous study (8), were associated using Pearson's correlation coefficient (r). The pairs creatinine/urea and urea/urate gave (r) values higher than 0.50, in the majority of patients, whereas the pair creatinine/urate gave (r) values symmetrically distributed around zero for all patients. This fact suggested independence between serum creatinine and urate in renal recipients during the stable period condition.

We established the diagnostic validity of significant changes in these quantities according to the evolution of a population of transplant patients.

Among the 75 renal transplant recipients included, two sub-groups were established:

1.- Fifty-seven patients who were clinically stable and showed no evidence of crisis for more than three years after transplantation (non-rejection group).

2.- Eighteen patients who suffered acute rejection after a short period of clinical stability lasting 2 to 5 weeks (rejection group). Rejection had been confirmed by clinical observation, analytical profile, Doppler ultrasound study in 7 patients and by histological study in 11 patients.

Permission for enrollment in the study was obtained from all patients, as required by the Helsinki II protocol.

Serum specimens were collected according to the standard hospital follow-up protocol designed by the nephrologists for renal transplant recipients (6,8).

Under this protocol the samples studied in the present work were drawn at different intervals of time, ranging from once per week to once per month (for the non-rejection group) (6).

The reference change values for creatinine and urate, at various probability levels and applied to a single quantity or the two quantities combined, were calculated according to the formula:

$$RCV = z_p (2)^{-} . (CV_{w+a})$$

where z_p is the covering factor for a certain probability, and CV_{w+a} is the within-subject biological plus analytical coefficient of variation.

If there is no correlation between two quantities in a subject then, under stable conditions, each quantity has less than a given probability for error when detecting changes, depending on the z_p selected (e.g. if the probability for one quantity to exceed the reference change is 5%, the probability of two quantities combined is 1/20 x 1/20 = 1/400 (0.25%)

The criteria commonly used to judge the diagnostic performance of a biochemical test are diagnostic sensitivity, diagnostic specificity, and predictive values of the positive and the negative tests (11,12).The diagnostic performance of the RCV value for creatinine and urate combined was calculated from the group of 75 patients studied. The *sensitivity* is the fraction of all true positive test values (TP) from post-transplanted patients that preceded an acute rejection and the *specificity* is the fraction of all true negative test values (TN) for all testings of all stable post-transplanted patients (without any results above the RCV assigned). The prevalence of acute rejection (n=18) in the patients deemed eligible for the study in our hospital (n=75) was 0.24.

The reference change values between creatinine and urate combined were calculated using the RCV formula described before. The application of this formula to the CV_{w+a} values for creatinine of 12.3% and urate of 13.5% (6) at 2.25% false-positive (FP) probability, (z_p=1.04), for two independent quantities combined gives RCV for creatinine of 18.1% and RCV for urate of 19.8% (Table 1) .if accepted or footnote to page 6).

Figure 1 (a,b) shows the percent differences between consecutive results for creatinine and urate found in the 57 patients from the non-rejection group and in the 18 patients from the rejection group. Horizontal and vertical boundaries represent the RCVs for creatinine and urate, respectively, using the predetermined theoretical FP probability of 2.25% (85% covering interval for each) .The triangle points represent consecutive combined differences higher than the respective RCVs, indicating FP and TP results respectively.

Bold points indicate TN and false negative (FN) results, respectively. In the figure 1a only one randomly chosen consecutive combined difference for each patient was plotted to simplify the figure.

In the figure 1b the empty points correspond to all remaining consecutive combined differences available for the rejection group of patients.

Table 2 shows sensitivity, specificity and predictive values for the prevalence of kidney rejection in the patients studied with a short period of stability in this study (0.24) at the 95% , 85% and 80% covering intervals.

According to the questionnaire directed to nephrologists (10) a >25% difference between consecutive creatinine determinations, when creatinine concentrations were outside of the reference interval was the relevant signal for impending crisis. The clinicians requested urate analysis to evaluate immunosupressor concentration but did not use it as an indicator of rejection.

When the specialists were asked for their opinions on the consequences of *false-negative prediction of crises,* their answers were related to patient safety and the cost of treatment. Regarding the patient, graft loss means a return to dialysis and this implies a high cost, both in discomfort to the patient and in the expense of dialysis.

The consequences of *false-positive prediction of crisis* were related to excessive immunosuppression, which has repercussions for the patient as physiological stress and susceptibility to opportunistic infections. Cost considerations depend on the immunosuppressor used and possible

complications due to infection. The clinicians emphasized that false positives have to be minimized.

We found that the RCVs of 18.1% for creatinine together with 19.8% for urate at an 85% covering interval showed the best combination of sensitivity, specificity, and positive and negative predictive values for the purpose of detecting potential rejections. When the 25% criterion used by the nephrologists was compared to this combined RCV criterion by applying to the population of 75 patients studied, we found that the number of rejections detected and false-negatives were the same (sensitivity of 0.722,confidence interval from 0.433 to 0.905, see table 2). However, 8 more false positive predictions were obtained with the >25% than with the combined RCV criterion. In the non-rejection group (n=57) 10 patients were falsely classified as experiencing rejection with the >25% criterion and 2 with the combined RCV criterion, giving specificities of 0.825 (0.677-0.916) and 0.965 (0.851-0.995) respectively.

- We found that when RCVs from creatinine and urate combined were used to predict crises, the false-positive probability was reduced and there was a considerable increase in diagnostic specificity.

The methodology used in this work pointed out the need to improve the laboratory reports (13):

- Include the RCV calculations for the combination of significant analytes for the pathology studied in the laboratory data processing system.
- Mark the test results showing a significant RCV with respect to the previous result.

· Include in the report a plot showing evolution of the analytes with a critical role in detecting changes in the pathology monitored.

A limitation of our RCV model to detect changes in the evolution of kidney graft recipients during monitoring is that it can only be applied to patients who have experienced a certain period of favorable clinical evolution. Regarding the usefulness of the model, we mention a few points. First, though it can only benefit patients who have achieved an interval of clinical stability, these are precisely the ones in whom surveillance may be more relaxed (the patient has recovered from the operation and feels better, analyses are less frequent) and a specific, objective biochemical marker could be of greatest value. Second, the constituents providing an early indicator of rejection are among those analyzed in the standard protocol and at exactly the same frequency. Thus, no additional cost, effort or discomfort to the patient is implied by the use of this approach (14).

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Table 1. Theoretical probabilities for FP for various z-values using one single test or both tests combined, when these are independent.

Creatinine (RCV,%)	*Urate (RCV,%)*	*Z (for increase only)*	*FP probability For 1 quantity*	*FP probability For 2 independent quantities combined*
28.6	31.4	1.65	5%	0.25 %
18.1	19.8	1.04	15%	2.25 %
14.6	16.0	0.84	20%	4.00 %

(Table1)

Table 2. Diagnostic validity for the patients (based on our prevalence of kidney rejection) for three covering intervals

Prevalence = 0.24 at	*95% covering interval*	*Confidence limits*
Sensitivity	0.278	(0.095 – 0.567)
Specificity	1.000	(0.904 – 1.000)
Positive predictive value	1.000	(0.239 – 0.994)
Negative predictive value	0.814	(0.760 – 0.879)
Prevalence = 0.24 at	*85% covering interval*	*Confidence limits*
Sensitivity	0.722	(0.433 – 0.905)
Specificity	0.965	(0.851 – 0.995)
Positive predictive value	0.867	(0.478 – 0.982)
Negative predictive value	0.917	(0.826 – 0.971)
Prevalence = 0.24 at	*80% covering interval*	*Confidence limits*
Sensitivity	0.778	(0.487 – 0.935)
Specificity	0.877	(0.738 – 0.950)
Positive predictive value	0.667	(0.370 – 0.856)
Negative predictive value	0.926	(0.820 – 0.979)

(Table 2)

Table 1. Theoretical probabilities for FP for various z-values using one single test or both tests combined, when these are independent.

Table 2 . Diagnostic validity for the patients (based on our prevalence of kidney rejection) for three covering intervals.

Figure 1 (a,b). Double Reference Change (non rejection and rejection groups), at 85% covering interval for each creatinine and urate.

Legend to Figure 1a

▼ FP results

● TN results

Legend to Figure 1b

▼ TP results

● FN results

○ remaining consecutive combined differences.

Footnote to page 6.

For z_p = 1.04, the tail area of distribution for one quantity is 15%. This means 3 false positive each 20 results. For two quantities combined, the probability is 3/20 x 3/20 = 9/400 = 0.0225. That is 2.25% probability.