

The value of different washout parameters in the evaluation of renal transplants by technetium-99m-ethylene-I-I-dicysteine

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Abstract

Technetium-99m-ethylene-I-I-dicysteine (^{99m}Tc-EC) is an agent with a potential for renal imaging. It is reported that EC uses the same tubular transporter system as ortho-hippurate (OIH) and mercaptoacetyltriglycine (MAG3) and that this agent has good imaging properties and higher renal clearance than MAG3. *In this study we used ^{99m}Tc-EC to compare different washout parameters in renal transplanted patients. Sixty nine scans in 55 patients (38 males, 17 females) were performed with ^{99m}Tc-EC during the follow-up period after kidney transplantation. After bolus injection of 280MBq ^{99m}Tc-EC, 60x1sec and 29x1min images were taken in anterior position. Perfusion of transplanted kidneys were examined visually and perfusion indices (PI) were calculated according to Hilson's method. The semiquantitative washout parameters such as 20 and 30min to peak activity ratios and 20 and 30min to 3min activity ratios were calculated. The patients were clinically evaluated by nephrologists experienced in renal transplantation cases and followed-up by serum creatinine and blood urea nitrogen determinants which were checked every postoperative day until stabilized to a optimal level for each patient. Two standard deviations above the mean values derived from all cases with normal functioning transplanted kidneys were calculated for each method. Then, these values served as the threshold to differentiate the pathological cases respectively. Of the 69 total studies performed, we found 34 normal kidneys, 14 rejections, 19 acute tubular necrosis (ATN) and 2 cyclosporin A toxicity cases. The number of abnormal cases detected with 30/3min, 30/max, 20/3min and 20/max indices were 27, 26, 24, and 18, respectively. *In conclusion, we strongly recommend in studying renal transplants to consider the activity at the 3rd min post injection as a reference point instead of the time to maximum activity for washout index calculation. If, for any reason the time to maximum counts using ^{99m}Tc-(peak activity) is preferred, then the period of study should not be performed for less than 30min to achieve reliable results.**

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Introduction

Complications such as acute tubular necrosis (ATN), acute rejection (AR) and cyclosporin-A toxicity (CyAt) occur at specific periods of time in the post-transplantation period. Ischemic damage leading to ATN is a possibility in the majority of cadaveric kidneys and is most frequently present at the time of transplantation. Most of the time it resolves without any treatment. Acute rejection occurs in the first weeks and quite often during the first 3 months after transplantation. It may also occur at any time even years after transplantation. Chronic rejection is a slow process with progressive arterial narrowing and deterioration of transplant perfusion ending in complete loss of function. Cyclosporin-A toxicity is seen while the patient is under medication and is often superimposed on other complications [1]. For the clinicians, it is important to differentiate between these complications in order to plan the appropriate treatment. Nuclear medicine tests for this purpose, measure renal function and play an important role in the differentiation of these complications.

Functional evaluation of renal transplants are divided into 3 categories corresponding to the classic three phases of the renogram namely, perfusion, extraction (parenchymal transit) and excretion of the transplanted kidney. The blood flow can be assessed both qualitatively and quantitatively by radionuclide angiography if a good bolus injection is given. The normalised fist pass curve can be determined by visual inspection to estimate whether the graft has a well defined flow peak or not. There are several methods of quantifying perfusion. The most commonly used is Hilson's perfusion index. For the evaluation of the second renogram phase, various indices or renal clearance of radionuclide agents have been calculated from the height, the slope or the area under time activity curve [2]. The tubular transit time can be calculated by deconvolution analysis using a parenchymal region of interest. It can be approximated by the whole kidney transit time or simply by the peak time. Changes in the mean transit time correlate with renal dysfunction [3]. The third phase of the renogram evaluates urine flow. In this phase, the

retained activity in the transplanted kidney is of concern. The distinction between cortical and pelvic retention of the radiopharmaceutical is important.

Parenchymal retention indicates vasomotor nephropathy, ATN or AR, while pelvic retention indicates various types of obstruction [3]. A quantitative measure of such retention is useful for monitoring the patient's course on sequential examinations. In this aspect, many different washout parameters were suggested in the past to evaluate the fate or functionality of transplanted kidneys. In this study, we overviewed the selected washout parameters, such as 20 and 30min to peak activity and 20 and 30min to 3min activity ratios in terms of finding the best suitable excretion parameter for evaluation of renal transplant patients. We used technetium-99m n-n ethylene-l-dicysteine ($^{99m}\text{Tc-EC}$), a radiopharmaceutical, which uses the same tubular transport mechanisms as of iodine-123 ortho-hippurate ($^{123}\text{I-OIH}$) or technetium-99m mercaptoacetyltriglycine ($^{99m}\text{Tc-MAG3}$).

Materials and methods

Sixty-nine renal scans in 55 patients (38 males, 17 females) were performed with $^{99m}\text{Tc-EC}$ during the follow-up period after kidney transplantation. We mostly received transplanted kidneys from related donors. About 1110MBq of $^{99m}\text{Tc-pertechnetate}$ were used to reconstitute with EC (Izinta, Hungary) according to the manufacturer's recommendation. The radiopharmaceutical was ready to use within 10min. The radiochemical purity of $^{99m}\text{Tc-EC}$ was checked randomly on every fifth study, by performing chromatography using Whatmann 4 chromatographic paper and acetone as solvent (system A) as well as using ITLC-SG and 0.5 Mol acetic acid as solvent (system B). The labeling efficiency was found satisfactory in all occasions. Using bolus injection of 280MBq $^{99m}\text{Tc-EC}$, 60 of 1sec and 29 of 1min images were acquired by a computer in the anterior projection. Regions of interest (ROI) were defined over the iliac artery opposite to the transplant, on the transplant parenchyma, the urinary bladder and on a crescent background area, selected lateral to the transplanted kidney. Normalised and background subtracted time activity curves were generated from these ROI. Perfusions of transplants were examined visually and perfusion indices (PI) were calculated according to well-accepted Hilson's method [4]. Normal range of PI was defined as 40%-150%. Using the same tomography curves, we calculated the semiquantitative parameters such as 20 and 30min to peak activity (20/max and 30/max) [5] and 20 and 30min to 3min activity (20/3min and 30/3min) ratios [6]. The patients were clinically evaluated by a nephrologist, who was experienced for renal transplantation follow-up by serum creatinine and blood urea nitrogen. In terms of these findings, patients were classified into four groups; normal kidneys, ATN kidneys, AR kidneys and kidneys with CyAt. Two standard deviations above the mean values of the above mentioned ratios derived from normal kidney population were accepted as a threshold. The same ratios were evaluated according to this level of threshold, for the other three groups. The consistency of the results of the above ratios were compared with the clinical conditions of the patients. Some selective cases were also histopathologically differentiated by using renal allograft biopsies and matched with the clinicians ultimate decision. The normal perfusion index and washout parameters were accepted as indicators of well

functioning kidneys and these patients were expected to have normal laboratory results. During the follow-up period which was on daily basis for the first week and monthly for three months after operation, the clinical course of the patients was examined in detail and radionuclide results were approved by the nephrologist who was involved in this study. Our study was approved by the local ethical committee and explained to the patients which gave their written consent.

Chi square test was used to find out the best excretion index to discriminate normal from abnormal kidneys. P values lower than 0.05 were accepted as statistically significant results.

Results

Of the 69 total scans performed, 34 were found to have normal kidneys (Fig. 1), 14 AR (Fig. 2), 19 ATN (Fig. 3) and 2 were diagnosed as CyAt cases. By using the 30/3min, the 30/max, the 20/3min and the 20/max indices, the numbers of abnormal cases detected were, 26, 24 and 18, respectively. Statistically there were no significant differences between the results of the 20/3min ratios as compared to the 30/3min, the 20/max and the 30/max ratios. The 30/max index also did not differ significantly from the 30/3min index in detecting pathologic cases ($P>0.05$). Nevertheless we found significant differences between the ratios of 20/max and 30/3min and the 30/max excretion indices ($P<0.05$) (Table 1). None of these ratios studied was capable to definitely discriminate between the rejected, necrotic or intoxicated kidneys.

Table 1. Comparison of different washout indices and corresponding statistical analyses

	20/3 m index	30/3 m index			20/3 m index	20/max index	
TP	24	27	51	TP	24	18	42
FN	11	8	19	FN	11	17	28
	35	35			35	35	

Chi-square: 0.65, P-value: 0.42 Chi-square: 2.143, P-value: 0.14

	20/3 m index	30/max index			30/3 m index	20/max index	
TP	24	26	50	TP	27	18	45
FN	11	9	20	FN	8	17	25
	35	35			35	35	

Chi-square: 0.28, P-value: 0.60 Chi-square: 5.04, P-value: 0.02

	30/max index	30/3 m index			20/max index	30/max index	
TP	26	27	53	TP	18	26	44
FN	9	8	17	FN	17	9	26
	35	35			35	35	

Chi-square: 0.08, P-value: 0.78 Chi-square: 3.92, P-value: 0.048

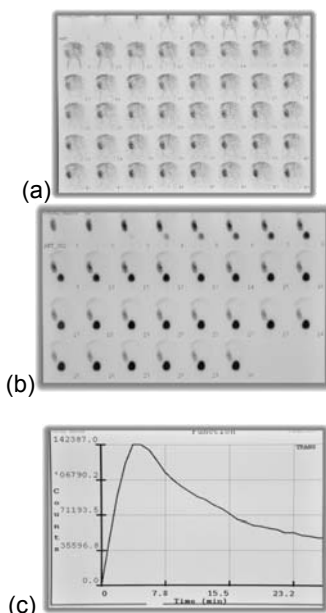


Figure 1. The perfusion (a) and functional (b) phase scintigrams and its corresponding time-activity curve (c) of a normal transplant study.

Discussion

Determining the function of renal transplants is an important early postoperative parameter to decide for continuing or discontinuing immunosuppression. For this purpose

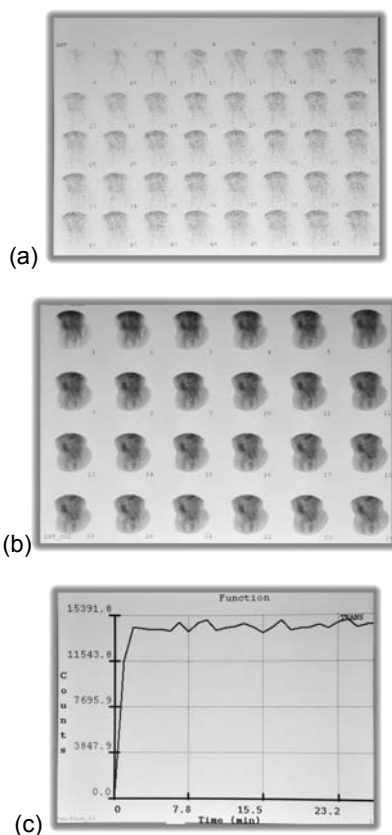


Figure 2. The perfusion (a) and functional (b) phase scintigrams and its corresponding time-activity curve (c) of a transplant with an acute rejection pattern.

On the other hand ^{99m}Tc -DTPA, while being a very good perfusion agent, is not capable to evaluate excretory function of the kidneys. New generation technetium labeled renal agents are ^{99m}Tc -MAG3 and later introduced radiopharmaceutical ^{99m}Tc -EC [8, 9]. All three phases of renal function perfusion, extraction and excretion can be indicated by ^{99m}Tc -MAG3 which is the most widely used renal agent at the moment. The advantage of ^{99m}Tc labeled agents are the high photon flux obtained from technetium which results in better count statistics as well as smooth lines in graphics in comparison with ^{131}I -OIH. Moreover, short half life and low radiation burden of technetium allows to make repeat studies, even in patients with severely reduced renal clearance, resulting in cortical retention of the radiopharmaceutical. Otherwise, using ^{131}I -OIH, it would not be practically possible to repeat the study within two days, because it could be necessary to administer higher doses several times to compensate for the long half-life of the retained ^{131}I -OIH.

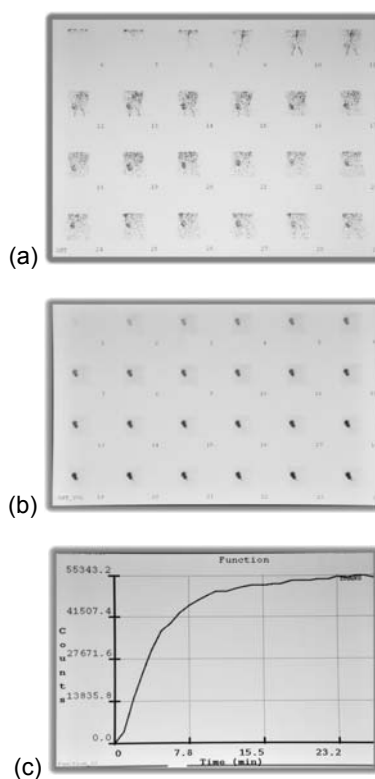


Figure 3. The perfusion (a) and functional (b) phase scintigrams and its corresponding time-activity curve (c) of a transplant with an acute tubular necrosis pattern. A similar pattern can be expected for those patients with cyclosporin toxicity.

Alternatively, to overcome these handicaps, the OIH labeled with ^{123}I could be used, but it would make the test more expensive to afford.

An alternative agent to ^{99m}Tc -MAG3 is ^{99m}Tc -EC. Animal and human studies in normal volunteers and renal recipients done with ^{99m}Tc -EC showed similar image qualities to those of ^{99m}Tc -MAG3 [10, 11, 12]. The behaviour of this tracer was closer to that of OIH than is the case with ^{99m}Tc -MAG3 [9]. It has been reported that the renal excretion characteristics of ^{99m}Tc -EC were superior to those of ^{99m}Tc -MAG3 [10]. Lower hepatobiliary excretion was also shown by ^{99m}Tc -EC, which enabled better delineation of the kidneys [13]. Moreover, ^{99m}Tc -EC could be prepared at room temperature with reproducible high labeling efficiency.

In the evaluation of the transplanted kidney, visual interpretation by observing the graft perfusion, tracer extraction and subsequent excretion of the radionuclide to the bladder is very important. However, it depends too much to the subjective decision of the physician and to image quality. For this reason semiquantitative analysis reported by different authors has been used in addition to visual interpretation. Others reported different methods for the evaluation of transplant perfusion [4, 14-16]. On the other hand quantitative measurements on sequential images of renal parenchymal retention were found very useful for monitoring the course of the patient. Excretion index has been used for this purpose [17, 18]. However, measurement of excretion index as burdensome, involving collection and counting of urine specimens and correction for post-voiding residual volume by using pre and post-voiding urine bladder images [6]. Simpler quantitative measurements of parenchymal retention have also been used either by calculating the ratio of background corrected renal counts at 20min to that of 3min [6] or by dividing the renal counts at 20min to those of maximum kidney counts [7]. In our transplanted patients we compared both methods of parenchymal retention to each other and evaluated if acquisition time longer than 20min will improve the diagnostic accuracy. For this purpose we also calculated the 30min/3min and 30min/max counts ratios. In previous studies it was shown that washout index can differentiate AR and ATN from normals but not AR and ATN from each other [6, 19]. Additional parameters such as perfusion and extraction (ratio of renal counts at 20min/background activity) are needed to make a differentiation between AR, ATN and CyAt. To our knowledge, there has been no other published study to compare this kind of excretion ratios in different clinical states of kidney transplants. In our study we searched for the best washout index differentiating abnormal cases (AR, ATN, CyAt) from normal transplants. According to our results, 30/max ratio separated pathological cases more efficiently than 20/max ratio (26 vs. 18 pathological transplants above normal threshold, respectively), which was found statistically significant ($P < 0.05$), indicating that longer acquisition times, when using maximum kidney counts as a time reference point, improve diagnostic accuracy (Fig. 4, 5). On the other hand statistical difference between the results of 20/3min and 30/3min ratios in the differentiation of pathologic cases from normals was not significant which means that the acquisition times longer than 20min are not needed when 3min counts are taken as a reference point to calculate washout of activity from the kidney (Fig. 6, 7). There was also no statistically significant difference between 20/3min and 30/max ratios. All these results bring us to a conclusion that the most appropriate washout index in evaluating transplant function is the 20/3min ratio.

Excretion index (EI) has been routinely used for a long time for measuring renal parenchymal retention. This index measures the fraction of activity removed from blood that persists in renal parenchyma but it is susceptible to the technical problems. The washout index 20/3min is a simpler but similar quantitative test because the activity in the kidney at 3min is proportional to effective renal plasma flow [6].

It has been shown that ^{99m}Tc -MAG3 and ^{99m}Tc -EC clearances are directly proportional to OIH clearance and thus to para-amino hippurate clearance [9, 13, 20]. For this reason, taking 3min as a reference point increases the accuracy of the test.

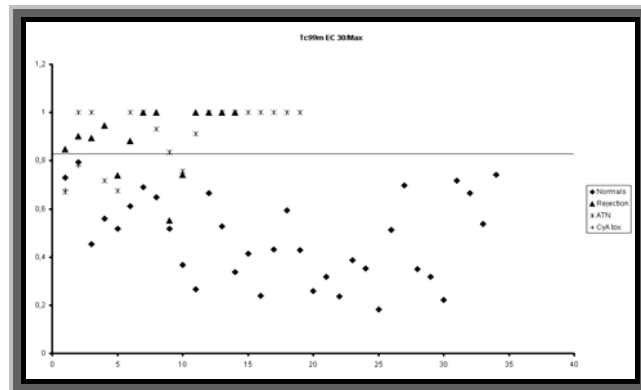


Figure 4. The distribution of patients in terms of pathologic cases ($n=26$) above the threshold line defined as mean + 2SD. (0.832) for 30/max index.

On the other hand when taking the 3min activity into account, the acquisition time of 30min instead of 20min,

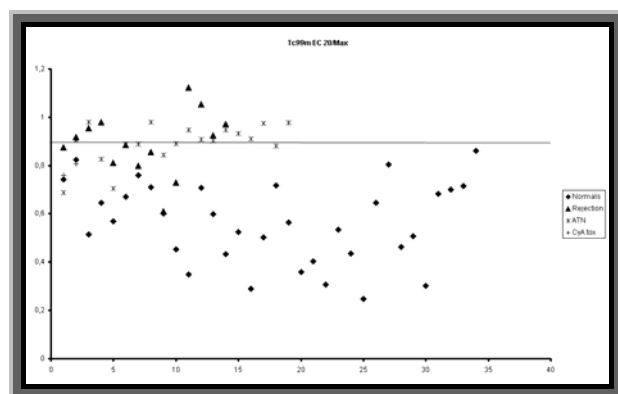


Figure 5. The distribution of patients in terms of pathologic cases ($n=18$) above the threshold line defined as mean + 2SD. (0.897) for 20/max index.

improves diagnostic accuracy only slightly (27 vs. 24 pathological transplants above normal threshold, respectively) without significant statistical difference (Fig. 6, 7).

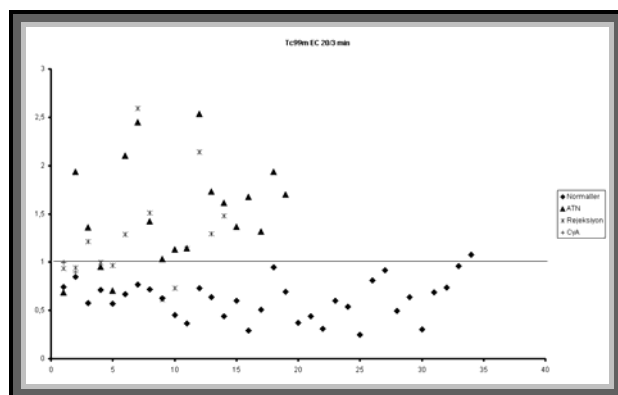


Figure 6. The distribution of patients in terms of pathologic cases ($n=24$) above the threshold line defined as mean + 2SD. (1.030) for 20/3 min index.

In conclusion, in the evaluation of parenchymal retention of ^{99m}Tc -EC in renal transplants using washout indices we strongly recommend to take the activity at 3rd min post injection as a reference time point instead of taking time to maximum activity into account if acquisition

is 20min. If, for any reason the time to maximum counts (peak activity) is preferred, then the period of study should not be less than 30min in order to achieve reliable results.

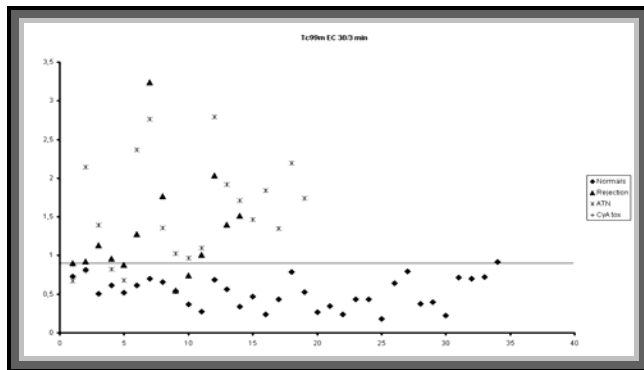
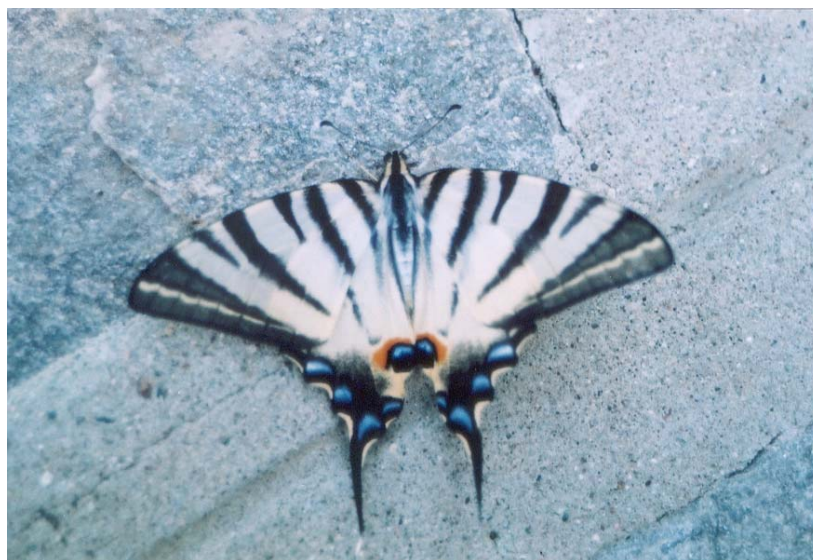


Figure 7. The distribution of patients in terms of pathologic cases (n=27) above the threshold line defined as mean+2SD. (0.917) for 30/3 min index.

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Butterfly Machaon. Machaon was one of the two sons of Asclepius, a physician himself who served and died at the Trojan war. Photographed by Professor I. Dokmetzioglou.