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A retrospective study of renal allograft pathology using the Banff 97 working classification

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Objective : *To determine the concordance of diagnosis in renal allograft biopsies by a semiquantitative measure (The Banff 97 working classification) compared with a descriptive terminology*

Setting : *Department of Pathology, Faculty of Medicine, Chulalongkorn University*

Research design : *A retrospective descriptive study*

Materials : *All cases of renal allograft biopsies that were available for review, pathologically and clinically, during 1986 to 1996.*

Methods : *Twenty renal allograft biopsies were available for review and reclassification, using the Banff 97 schema with blinded clinical information, laboratory data, and previous interpretation of the same biopsies. The slides were prepared with at least one slide for each stain, namely hematoxylin and eosin (H&E) stain, periodic acid-Schiff (PAS) stain, silver (Jone's) stain, and Masson trichrome stain. The biopsies that were unfit by the Banff criterion for minimal specification or adequacy were excluded.*

Result : *Only twelve cases of twenty were sufficient according to the Banff criterion. The findings showed one case of borderline change, one*

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of grade IA, two of IB (corresponding with cellular rejection), 4 of IIA, 1 of IIB and 3 of III (vascular rejection). This reclassification compared with the original diagnosis in descriptive terminology shows 25 % (3 cases) discordance. All cases had clinical data questionably diagnostic of rejection.

Conclusion : *Two of the three cases of discordance present in this study resulted from the difference in recognition of the presence of inflammatory cells that infiltrate the tubules and interpretation of the mild degree of the vascular change. Such lesions might have failed to be interpreted or over-read as mild tubulitis and intimal arteritis due to different pathologists. The other case of discordance that was over-read in the original report was caused by the interpretation of vascular change in the Immunofluorescence study. The prognostic value was undetermined in this study due to the lack of follow-up clinical data. However, many studies have shown that the Banff schema not only provide a semiquantitative measurement in making the diagnosis of acute rejection, but also predict the prognosis of rejection reversal. Because rejection is an inflammatory process that occurs with a focal or patchy character, the appropriate specimen as suggested by the schema together with clinical data are the most important data to ensure that the closest diagnosis to the existent pathology in the patient is attained.*

Key words : *Renal allograft rejection, Banff working classification.*

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วัตถุประสงค์ : *เพื่อศึกษาความสอดคล้องและความแตกต่างของการวินิจฉัยพยาธิสภาพของไต ที่ปลูกถ่ายในผู้ป่วยโดยอิงเกณฑ์ Banff 97 working classification ซึ่งเป็นการวินิจฉัยในเชิงกึ่งปริมาณ เปรียบเทียบกับการวินิจฉัยในเชิงพรรณนา*

สถานที่ทำการศึกษา : *ภาควิชาพยาธิวิทยา คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย*

รูปแบบการวิจัย : *ย้อนหลังเชิงพรรณนา*

ตัวอย่างที่ทำการศึกษา : *ชิ้นเนื้อไตที่ปลูกถ่ายทุกรายที่ส่งตรวจทางพยาธิวิทยาในช่วงปี พ.ศ. 2529 ถึง 2539*

วิธีการศึกษา : *รวบรวมชิ้นเนื้อไตที่มีประวัติผู้ป่วยได้รับการปลูกถ่ายซึ่งมีบันทึกแยกไว้ได้ทั้งหมด 20 ราย นำมาศึกษาจุลพยาธิสภาพอีกครั้ง จัดหมวดหมู่ และแบ่งกลุ่มโดยใช้เกณฑ์ Banff 97 โดยปราศจากข้อมูลทางคลินิกและข้อมูลเกี่ยวกับการวินิจฉัยที่ผ่านมา ชิ้นเนื้อเหล่านี้ได้ผ่านกรรมวิธีและการย้อมซึ่งจำเป็นในการดูพยาธิสภาพของไตตามปกติ ชิ้นเนื้อที่มีจำนวนของ glomerulus และ หลอดเลือดไม่เพียงพอต่อการศึกษาได้ถูกคัดออก*

ผลการศึกษา : *จากจำนวนชิ้นเนื้อทั้งหมด 20 ราย มีเพียง 12 ราย ที่ผ่านเกณฑ์กำหนดปริมาณชิ้นเนื้อขั้นต่ำ ซึ่งเมื่อนำมาจัดแบ่งตามกลุ่ม พบว่ามี 1 ราย อยู่ในกลุ่มสงสัยภาวะ acute rejection อีก11 รายมีภาวะ acute rejection ซึ่งใน 11 รายนี้แบ่งย่อย ออกเป็น ระดับ IA จำนวน 1 ราย ระดับ IB 2 ราย ระดับ IIA 4 ราย ระดับ IIB 1 ราย และระดับ III ซึ่งมีการอักเสบทำลายของผนังหลอดเลือดอย่างรุนแรง 3 ราย ทุก ๆ รายได้รับการวินิจฉัยทางคลินิกว่าสงสัยภาวะ rejection และเมื่อศึกษาเปรียบเทียบกับการวินิจฉัยดั้งเดิม ซึ่งเป็นการวินิจฉัยเชิงพรรณนา พบว่ามีอยู่ 25 % ที่การวินิจฉัยไม่สอดคล้องกัน*

สรุป : *2 ใน 3 รายของผู้ป่วยที่การวินิจฉัยแตกต่างกันเนื่องมาจาก ความแตกต่างระหว่างผู้ให้การวินิจฉัย ในการตัดสินว่ามีการสอดแทรกของเม็ดเลือดขาวอยู่ระหว่างเซลหลอดฝอยไตหรือไม่ และมีการเปลี่ยนแปลงของผนังชั้นในสุดของหลอดเลือดขนาดกลางหรือไม่ ซึ่งมักมีปัญหาเมื่อพบการเปลี่ยนแปลงเพียงเล็กน้อย ส่วนอีก 1 ราย ที่การวินิจฉัยได้ผลแตกต่างกัน เป็นเพราะการพบการเปลี่ยนแปลงของหลอดเลือดขนาดกลางจากการย้อมทางอิมมูโนด้วยสารเรืองแสง ในการศึกษานี้ไม่ได้ศึกษาถึงความสัมพันธ์ระหว่างพยากรณ์โรคกับพยาธิสภาพในระดับต่าง ๆ เพราะขาดข้อมูลของผู้ป่วยภายหลังการวินิจฉัย อย่างไรก็ดี การแบ่งแบบ Banff มีเกณฑ์ที่ชัดเจนและละเอียดมากกว่าแบบดั้งเดิม ได้มีผู้ทำการศึกษาหาความสัมพันธ์ระหว่างพยากรณ์โรคกับพยาธิสภาพที่พบตามเกณฑ์ Banff schema ไว้ และพบความสัมพันธ์ของคะแนนตามความรุนแรงของพยาธิสภาพ โดยเฉพาะ การอักเสบของหลอดเลือดกับการหายของภาวะ rejection เนื่องจากธรรมชาติของภาวะ rejection มักเกิดเป็นแห่ง ๆ ดังนั้นการวินิจฉัยที่จะให้ผลสอดคล้องกับพยาธิสภาพที่มีอยู่จริงในไตที่ปลูกถ่ายนั้น จำเป็นต้องได้ปริมาณเนื้อเยื่อไตที่เพียงพอ ร่วมกับข้อมูลทางคลินิกของผู้ป่วย*

To determine the function of a renal allograft with accuracy, renal biopsy interpretation has become the most reliable measure, when combined with clinical Criteria. [1] Pathologists have been trying to make use of allograft biopsies to give as much information as possible. In 1976, before cyclosporine was introduced in clinical use, Finkelstein et al presented a classification of acute renal allograft rejection by grading the severity of interstitial inflammation, glomerulitis, and arteritis as a whole. [2] Banfi et al also published a comparable classification that recognized the features that suggest irreversible forms of rejection, infarction and extensive parenchymal necrosis. [3] Matas et al introduced an eight-graded system in 1983. This schema was divided into minimal-to-severe tubulointerstitial nephritis (the first four categories), minimal-to-moderate vasculitis (category five to seven) and category eight for cases with severe vascular rejection with fibrinoid necrosis. [4] The Banff 97 schema used in this study was developed from a working classification that originated in the meeting held in Banff, Canada on August 2 to 4, 1991 which was published in 1993 and refined in subsequent meetings every two years . [5,6] This current schema was the result of the Fourth Banff Conference on Allograft Pathology where pathologists using the Banff schema and those using the Collaborative Clinical Trials in Transplantation (CCTT) modification met with clinical investigators. [7] The list of diagnostic categories of the Banff 97 schema and the descriptive terminology [8] are shown in Table 1 and 2.

Table 1. Banff 97 diagnostic categories for renal allograft biopsies.

1. Normal
2. Antibody-mediated rejection

Rejection demonstrated to be due, at least in part, to anti-donor antibody

- A. Immediate (hyperacute)
- B. Delayed (accelerated acute)

3. Borderline changes: "Suspicious" for acute rejection
4. Acute/active rejection
   - Grade IA Cases with significant interstitial infiltration
   - IB Cases with significant interstitial infiltration and foci of severe tubulitis
   - IIA Cases with mild to moderate intimal arteritis
   - IIB Cases with severe intimal arteritis comprising >25% of the luminal area
   - III Cases with "transmural" arteritis and/or arterial fibrinoid change and necrosis of medial smooth muscle cells
5. Chronic/sclerosing allograft nephropathy
   - Grade I Mild interstitial fibrosis and tubular atrophy without (a) or with (b) specific (mild) changes suggesting chronic rejection
   - Grade II Moderate interstitial fibrisis and tubular atrophy (a) or (b)(moderate)
   - Grade III Severe interstitial fibrosis and tubular atrophy and tubular loss (a) or (b) (severe)
6. Other Changes not considered to be due to rejection

Table 2. Descriptive terminology in pathologic diagnosis of renal allograft rejection.

1. Hyperacute rejection
2. Acute rejection
   - Acute cell-mediated interstitial rejection
   - Acute vascular rejection
3. Chronic rejection

Materials and Methods

All renal allograft biopsies in Chulalongkorn Memorial Hospital between 1986 to 1996 were obtained from surgical files of kidney biopsies. Only twenty cases were available for pathological review. This was performed by one author (Kittikowit W.). Some of these materials were restained and recut by the other author (Amornsirivat V.).

Some of these specimens had been fixed in 10 % formalin and the others in Zenker's solution before they were process and embeding in paraffin blocks this resulted in variation in the tissue quality from case to case. Each section was cut at 2 to 3 μm. At least four slides were provided in four stains; hematoxylin and eosin (H&E), Periodic Acid Schiff (PAS), silver (Jone's), and Masson Trichrome. These biopsy specimens were reviewed and reclassified using the Banff 97schema[7] without clinical data or information of the original diagnosis. Once the review of the pathological findings and reclassification were accomplished, review of the clinical data from the pathological request forms and original diagnoses were performed. Table 3 displays the approximated relationship between the Banff grades of acute rejection and the descriptive terminology in the original diagnosis.

Table 3. Approximate relationship between the Banff grade of acute rejection and descriptive terminology.

Banff Grade	Descriptive Terminology
IA	Cellular rejection
IB	Cellular rejection
IIA	(Early/mild) vascular rejection
IIB	Vascular rejection
III	Vascular rejection

Results

Of the twenty biopsies, eight were regarded as insufficient for diagnosis and were excluded from the study, using the Banff 97 criterion.[7] The reclassification of the remaining cases using the Banff schema is presented in Table 4. The original reports were classified into three categories (Table 5). The unspecified category consisted of acute rejection, subacute rejection and chronic tubulointerstitial nephritis. Subclassification of these terms was determined by the details described in the microscopic

Table 4. Diagnostic categories and grades of acute rejection using the Banff schema.

Banff grade	No. of cases
Normal	0
Borderline	1
IA	1
IB	2
IIA	4
IIB	1
III	3
Total	12

Table 5. Diagnostic categories of acute rejection using the descriptive terminology.

Descriptive diagnosis	No. of cases
Normal	0
Cellular rejection	2
Vascular rejection	6
Unspecified	3
Others**	1
Total	**12**

* The diagnostic term used are acute, subacute rejection and chronic tubulointerstitial inflammation. The details of each case were provided in the microscopic findings.

** Chronic tubulointerstitial inflammation

findings of the original reports (Table 6). With these findings, compared with the original diagnosis in the descriptive terminology, we found three cases (25 %) that were discordant and nine cases (75 %) concordant in the acute rejection aspect. Two of the discordant cases were under-read by the descriptive terminology, and the other case was over-read (Table 7). The other findings are presented in Table 8. For all cases, biopsy was performed to rule out rejection. In three of these cases, cyclosporine toxicity was also suspected and obstructive uropathy was considered one case. The current review and the previous diagnoses showed some evidence related to the obstructive uropathy in the suspected case (acute interstitial nephritis and dilatation of some tubules). The serum creatinine level was matched case by case and the median for each Banff category is demonstrated in Table 9.

Table 6. Correlation of the unspecified category and the descriptive terminology.

Original terms	Reclassified descriptive terms	No. of cases	Banff grades
Acute rejection	Cellular rejection	1	IB
	Vascular rejection	1	III v3
Subacute rejection	Vascular rejection	1	IIA

Table 7. Comparison between the Banff schema and the descriptive terminology of the three cases of discordance.

Banff categories	Descriptive terminology	
Borderline change	Chronic tubulointerstial inflammation	[under – read]
IB	Acute vascular rejection	[over – read]
IIA	Acute cellular rejection	[under – read]

Table 8. Other finding additional to the acute rejection.

Findings	No. of cases
Chronic allograft nephropathy	6
Acute tubular necrosis	2
Acute interstitial nephritis	1

Table 9. Matched median of level of serum creatinine to the Banff grade.

Banff categories	Serum creatinine (mg %)
Borderline	2.9 (1)*
grade IA	3.38 (1)
grade IB	8.3 (2)
grade IIA	5.46 (4)
grade IIB	_ **
Grade III v3	7.98 (2)

* Show the number of cases that had the clinical data

** No clinical data of the only one case of this grade

Discussion

This semiquantitative study of the twelve graft biopsies shows a diagnostic concordance of 75 % between the Banff grading and the original diagnosis using descriptive terminology. However, the predictive valve of these biopsies cannot be evaluated in this study due to the lack of the follow-up clinical data. The 25 % discrepancy was the result of two under-read (16.7 %) and one over-read cases (8.3 %). This outcome is in contrast to the previous study of Dean et al. [9] The study showed the lowest degree of concordance befalling between Banff borderline and grade I rejection and the greatest degree of discrepancy was in normal and grade III. Moreover, the percentage of the over-read exceeded the under-read cases, 85.7 to 14.3. But in this study, the difference resulted from the demonstration of few mononuclear cells in few tubules and the interpretation of the intimal change of one intralobular artery that lead endothelitis to be considered. This outcome was effected by the difference in interpretation of the different pathologists. The use of a panel of examiners may help in solving this problem. The other discordant case that was over-read resulted from vascular change that was demonstrated in the immunofluorescent studyThe criterion used in this study did not mention the application of this test.[5-7] However, the term over-read and under-read used in this study are based on the Banff classification that could misinterprete because there was no goal standard use in evaluation currently. Dooper et al [10] have proposed that the Banff classification has a tendency to over- diagnose acute rejection for this reason.

Some studies have demonstrated that paired renal biopsies increase the sensitivity in detection of rejection. [11,12] The Banff schema also suggests the biopsy should contain at least 10 glomeruli and two arteries. However the threshold for a minimum sample in general interpretation of renal biopsies is seven glomeruli and one artery. Moreover, two separated cores containing cortex or two separated areas of cortex in the same core are recommended. [7]

The median of the serum creatinine level in our study does not help in prediction of the severity of the rejection due to the absence of a correlation with each grade. This might relate to the small sample size. Gaber et al. [13] found a correlation between serum creatinine level and rejection reversal. Gaber et al. [13,14] also published a correlation of the Banff sum score

with the reversibility of rejection and responsiveness to steroid therapy. This study also pointed that the vascular and glomerular scores were significantly higher in severe rejection and the high vascular score alone implied irreversible rejection with steroid therapy.

The discordant case that was classified as borderline in this study also showed chronic allograft nephropathy grade I (mild) while the original diagnosis was of chronic tubulointerstitial nephritis. Although interstitial inflammation with very mild degree of tubulitis is not counted as an acute rejection condition, there is some relationship between the two. According to the study of Meehan et al, [15] subsequent acute rejection occurred in 28 % of cases that were formerly diagnosed as borderline and in whom no additional anti-rejection therapy was administered. Because acute rejection is an inflammatory process that occurs in a focal or patchy fashion, so the morphologic examination alone cannot determine the behavior of a borderline infiltrate selectively within a vascular lesion. [12,16,17] The molecular study of D'Elios et al [18] and the immunohistochemical study of Kajawara et al [19] found the difference of the cytokine production between the cases of borderline infiltrate and acute rejection. The studies showed that the production of Interferon-γ (IFN-γ) and granulocyte-macrophage colony-stimulating factor (GM-CSF) were much higher in acute rejection cases, three fold greater of the former and demonstrable immunohistochemical expression of the latter. However, the specific roles of these cytokines in graft function are still inexplicable.

In conclusion, the Banff classification provided the finer guidelines in determining severity of rejection. This information leads to the judgement in determining the immunosuppressive protocol that suits to each patient.

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