第3回　腎臓移植の基準等に関する作業班

議事次第

日時：平成22年10月25日（月）
10：00～12：00
場所：厚生労働省　共用第8会議室

1．開　会

2．議　事
（1）レシピエント選択基準について
＊シミュレーション結果について　＊リンパ球交叉試験について
（2）その他
3．閉　会

〈配布資料〉
資料1－1　腎臓移植希望者（レシピエント）選択基準について
資料1－2　腎臓移植希望者（レシピエント）選択基準シミュレーションの状況
資料2　腎臓移植希望者（レシピエント）選択基準（修正案）

参考資料1　腎臓移植希望者（レシピエント）選択基準の運用状況について
（社団法人日本臓器移植ネットワーク提出資料）
参考資料2　腎臓移植希望者（レシピエント）選択基準（現行）
参考資料3　腎臓器提供者（ドナー）適応基準（現行）
参考資料4　HLA に関わる選択基準に関する提言
（日本移植学会・日本組織適合学会共同作業部会提出）
参考資料5　Flow cytometry　crossmatch に関する文献
参考資料6　HCV 抗体陽性ドナーに関する文献

資　料　1－1

腎臓移植希望者（レシピエント）選択基準について

1. 経緯

平成7年に制定された腎臓移植希望者（レシピエント）選択基準については、阻血時間の短縮のため、都道府県内配分を中心とすること、及び小児患者並びに長期待機患者の優先度を上げることなどを考慮し、平成14年1月に選択基準の改正を行った。

その後、平成21年7月の「臓器の移植に関する法律の一部を改正する法律」の成立を踏まえ、平成22年1月、選択基準における親族への優先提供に関する規定を定めた。

（改正の議論）

平成13年	2月	第1回臓器移植委員会（腎臓移植の現状について議論）
	5月	腎臓移植に関する作業班において議論（第1～5回）
	12月	第5回臓器移植委員会（改正案について了承）
平成14年	1月	選択基準の変更 ～新たな基準で運用
平成21年	11月	第1回腎臓移植の基準等に関する作業班において議論
平成22年	1月	選択基準の変更 ～新たな基準で運用

2. 検討のポイント

第2回作業班（平成22年8月26日開催）で出された主な論点は以下のとおり。

1）　待機日数の延長

平均待機日数：旧基準では2,467日、現行基準では5,208日

2）　16歳以上の若年者への配分が少ないこと

現行基準の運用開始以降提供された1,327例の腎移植の内、16歳未満は約8%（88例）、16歳から20歳未満はゼロ、20歳代は0.9%（12例）

資　料　1－2

腎臓移植希望者（レシピエント）選択基準改訂

に係るシミュレーションの状況

1　シミュレーションの前提条件

＊　ドナー条件：現行基準で行われた脳死下での提供事例 30 例

＊　待機患者条件：平成 22 年 10 月 13 日現在の待機患者 11,708 名

2　シミュレーションの方法

下記の条件ごとに、レシピエント候補者を選択し第 1 位及び第 2 位につき、検討した。

A　：現行基準

B　：現行基準から、HLA の点数を 1.15 倍※1とした。

C-1：待機日数の配点を概ね半減し※2、小児の年齢加点を 0 とする。

C-2：待機日数の配点を概ね半減し※2、年齢加点を、
「16 歳未満：8 点、16 歳～20 歳未満：4 点、20 歳台：2 点」とする。

C-3：待機日数の配点を概ね半減し※2、年齢加点を、
「16 歳未満：12 点、16 歳～20 歳未満：6 点、20 歳台：3 点」とする。

※1　現行基準で行われた脳死下での腎提供事例 80 例について、レシピエント選択リストを作成し、そのリストの第 1 位のレシピエント 80 名の所在地、HLA、待機日数の平均換算点数の比は概ね 1.15：1：1.15 である。

※2　待機期間が 10 年までは 0.5 点／年、11 年～20 年までは 0.25 点／年、20 年以上は 0.125 点／年となるような近似値を log 式とする。（別紙1参照）

3　現行基準の分析（別紙2）

レシピエント選択時における患者本人の意思確認等の影響を除去するため、過去 80 例（脳死提供事例 20～102 例目まで：腎選定が行われなかったものを除く）の提供事例において、第1位にリストアップされた患者の分析を行った。

提供事例 80 例の実際の第一位患者 80 名

平均点　　：所在地：11.4 点　HLA：9.98 点　待機期間：11.49 点

平均待機日数：5412.1 日

16 歳未満が第一位：16 名（20%）

4 シミュレーションの結果概要(別紙3)

	A	B	C-1	C-2	C-3	実績＊	3の分析
平均待機日数(日)	5579.1	5610.4	5091	4718.1	3529.1	5207.9	5212.1
うち16歳以上(日)	6357.3	6309	5091	4883.2	4767	5521	6389.7
16歳未満　人(%)	9 (15)	8 (13)	0	3 (5)	20 (33)	88 (8)	16 (20)
16歳から20歳未満	0	0	0	0	1(2)	0	0
20歳台	0	0	0	8 (13)	8 (13)	12 (0.9)	1 (1.3)
待機期間10年未満、16歳以上の候補者数　人(%)	0	3 (5)	7 (12)	5 (8)	14 (15)		3(3.7)

＊実績は参考資料　1を参照

A,B,C:n=60　3の分析:n＝80　実績:n=1327

資　料　2

腎臓移植希望者（レシピエント）選択基準（修正案）

赤字が修正（追加）部分

１．前提条件

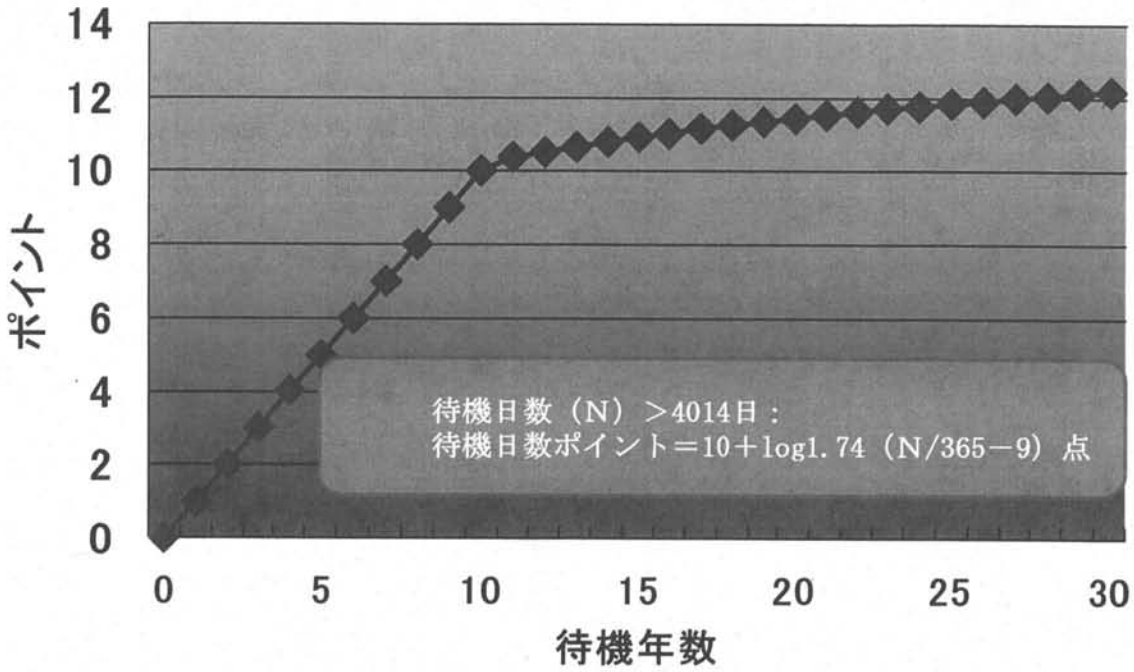
（２）リンパ球直接交叉試験（全リンパ球又はＴリンパ球）陰性
なお、リンパ球交叉試験はFlow cytometry等の高感度方法を用いて行うことが望ましい。

３．具体的選択法

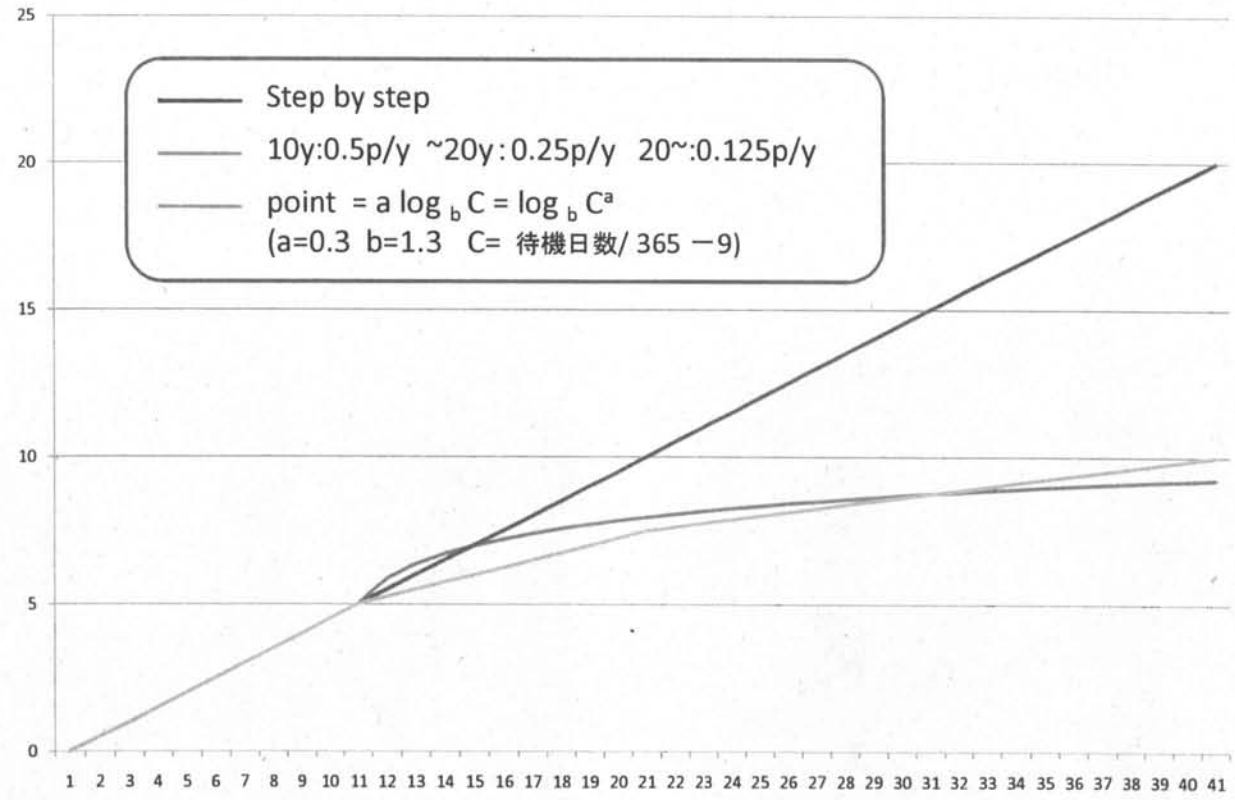
（３）２．の（１）～（４）の合計点数が高い順とする。ただし、これらの条件が同一の移植希望者（レシピエント）が複数存在した場合には、臓器搬送に要する時間、医学的条件に配慮する。
また、PRA検査は可能であれば行うこととする。なお、PRA検査はFlow cytometry等の高感度方法を用いて行うことが望ましい。

別　紙　1

現行基準の待機期間とポイント

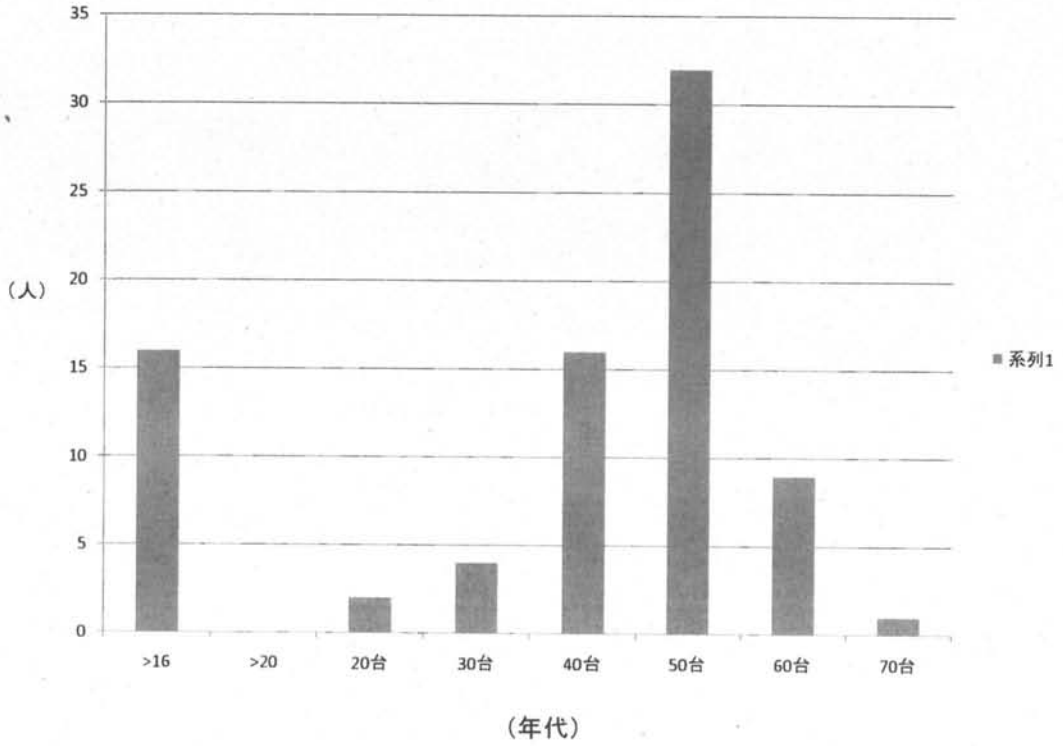


腎レシピエント待機日試算

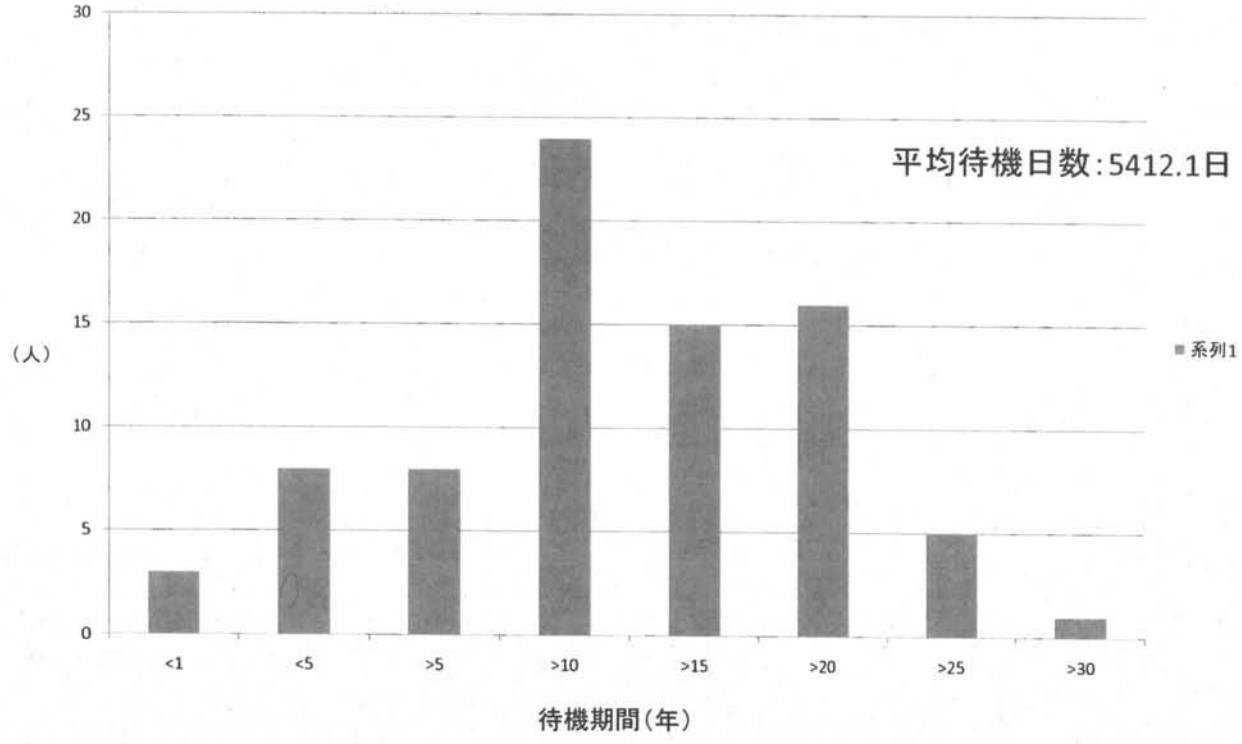


別　紙　2

脳死提供80例レシピエント候補第1位の年齢分布（N=80)

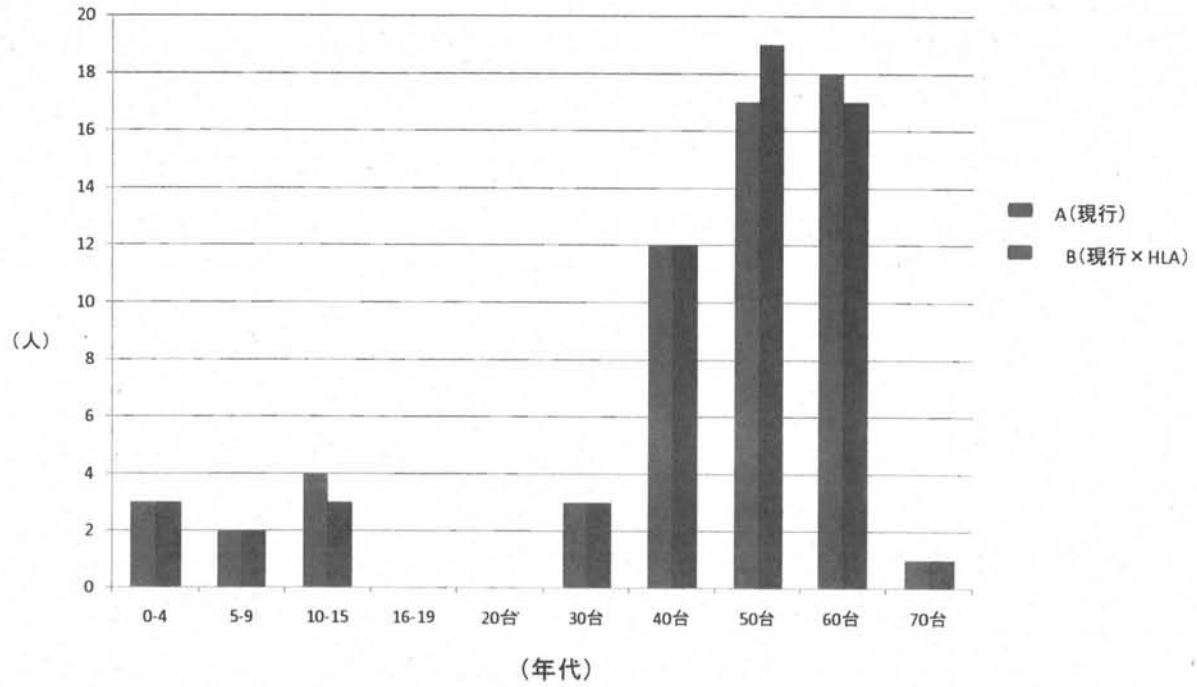


脳死提供80例レシピエント候補第1位の待機期間分布（N=80)

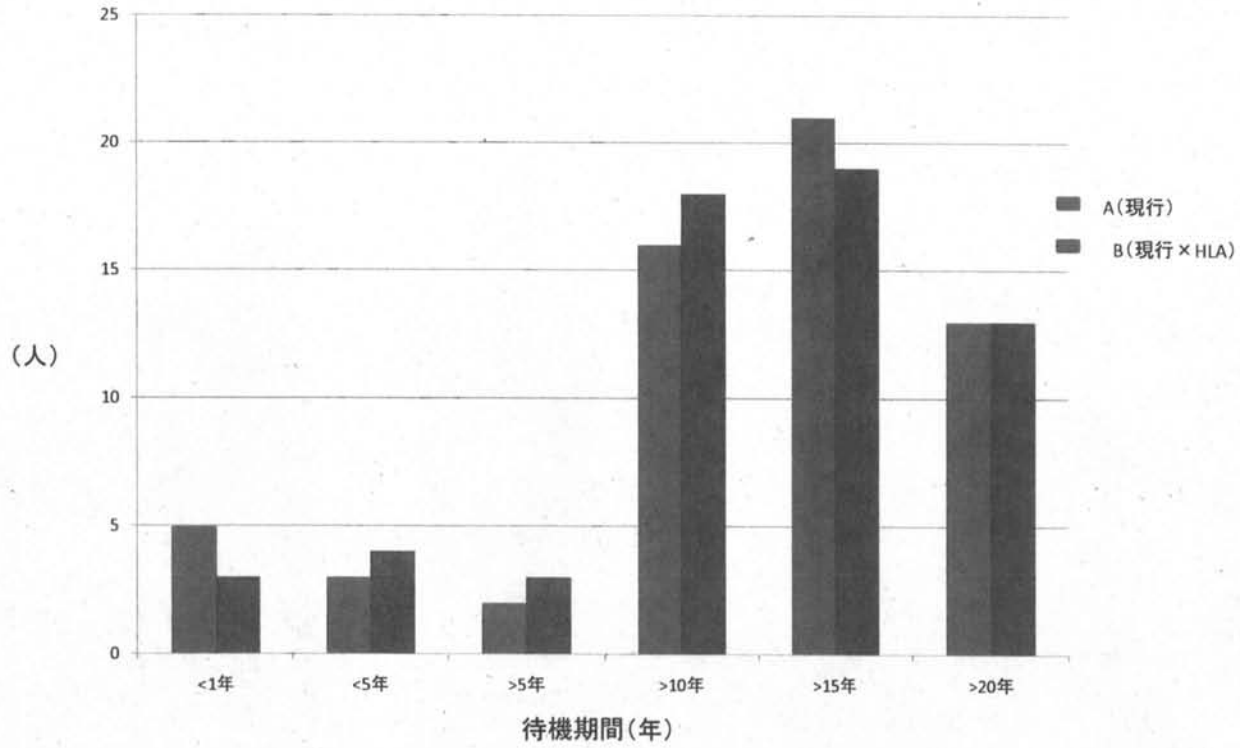


別　紙　3

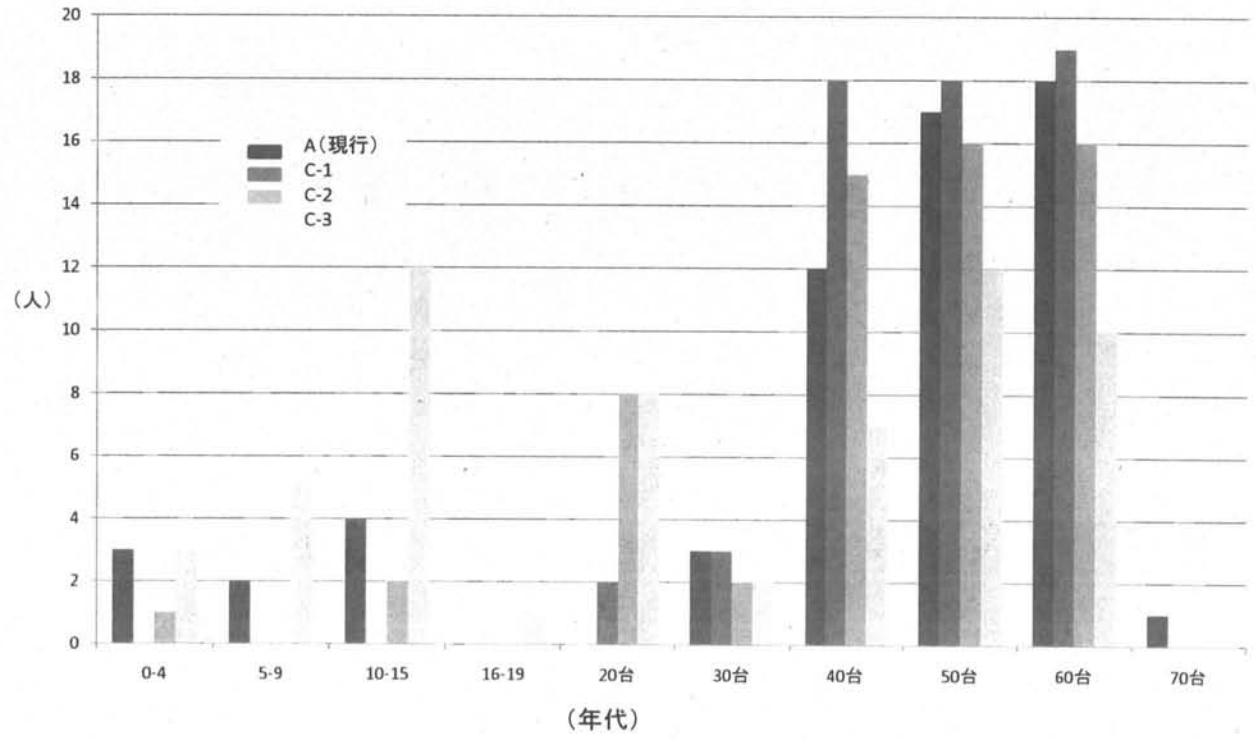
現行基準（A）とHLAの補正（B）の比較（年齢分布）N=60



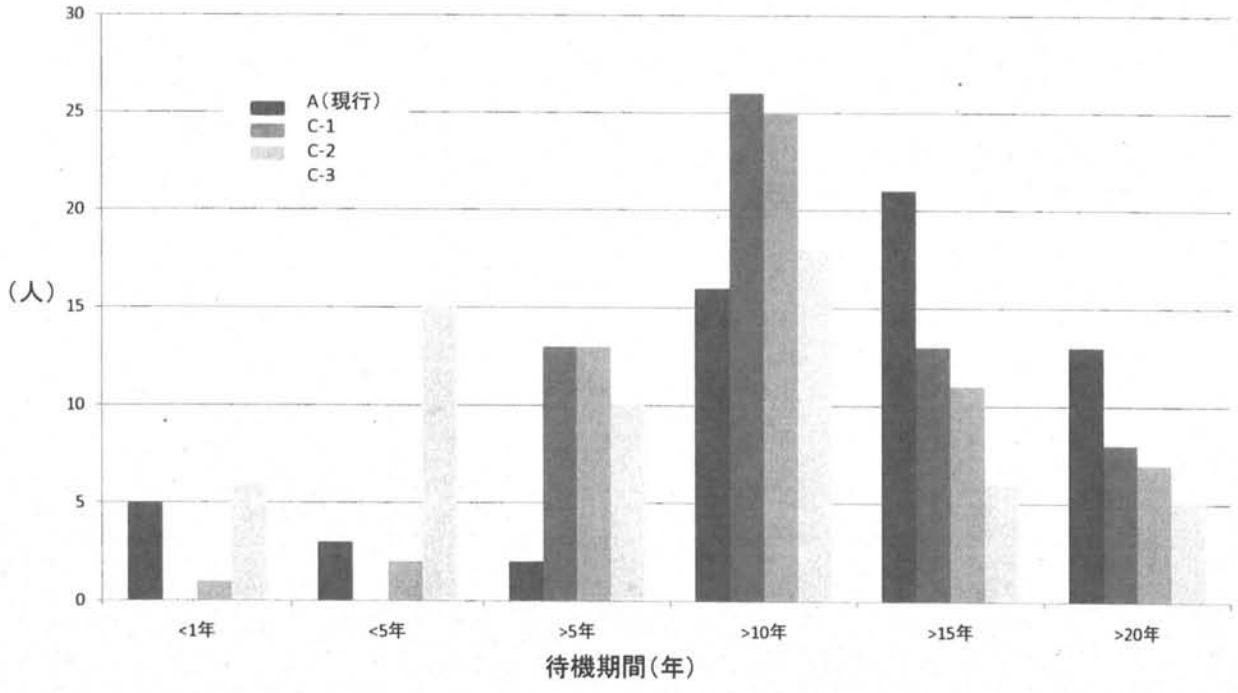
現行基準（A）とHLAの補正（B）の比較（待機期間分布）N=60



現行基準(A)と試算待機日数(C)による選定比較(年齢分布)N=60



現行基準(A)と試算待機日数(C)による選定比較(待機期間分布)N=60



参考資料 1

腎臓移植希望者(レシピエント)選択基準の運用状況について
(社団法人日本臓器移植ネットワーク提出資料)

レシピエント選択基準変更前後のシッピング

旧基準　(1995.4.1 ～ 2002.1.9　N=1,063)
新基準　(2002.1.10～2009.12.31　N=1,327)

	同一県内	ブロック内 県外	ブロック外	小児
旧基準	29.0 %	58.9%	12.1 %	2.7 %
新基準	81.5 %	18.3%	0.2%	6.6%

レシピエント選択基準変更前後の
HLA不適合抗原数・ドナー年齢・阻血時間

旧基準　(1995.4.1 ～ 2002.1.9　N=1,063)
新基準　(2002.1.10～2009.12.31　N=1,327)

	HLA不適合抗原数 (検索型)		ドナー 年齢	温阻血 時間	総阻血 時間
	DR	AB		(分)	(分)
旧基準	0.11 ± 0.34	1.28 ± 0.98	45.44 ± 17.11	7.94 ± 10.85	861.09 ± 402.95
新基準	0.51 ± 0.54	2.17 ± 0.97	48.94 ± 15.65	7.19 ± 8.97	731.01 ± 359.00
	(P<.001)	(P<.001)	(P<.001)		(P<.001)

レシピエント選択基準変更前後の
レシピエント年齢・待機期間・透析期間

旧基準　（1995.4.1 ～ 2002.1.9　N=1,063）
新基準　（2002.1.10～2009.12.31　N=1,327）

	レシピエント年齢（全体）	レシピエント年齢（16歳以上）	待機期間（年）	透析期間（年）
旧基準	44.60 ± 11.22	45.56 ± 9.80	6.76 ± 4.86	10.12 ± 6.21
新基準	47.44 ± 12.88	50.05 ± 8.58	14.27 ± 5.37	17.24 ± 6.70
	(P<.001)	(P<.001)	(P<.001)	(P<.001)

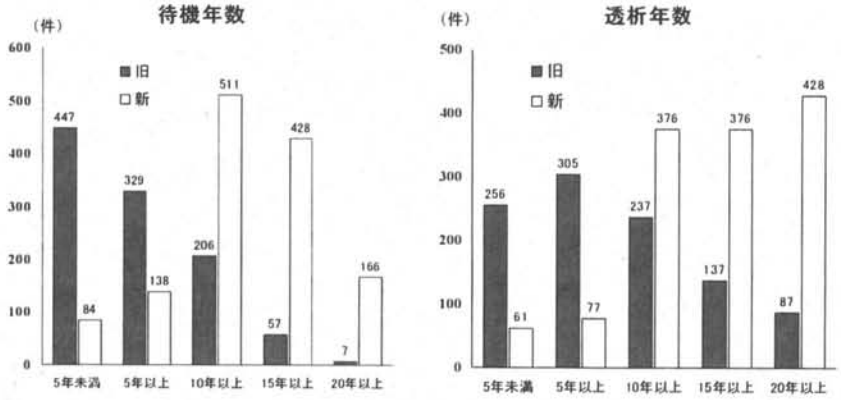
レシピエント選択基準変更前後の
レシピエント待機日数・透析日数

旧基準　（1995.4.1 ～ 2002.1.9　N=1,063）
新基準　（2002.1.10～2009.12.31　N=1,327）

		待機日数	透析日数	
旧基準		2467.04±1772.57	3694.26±2265.90	(P<.001)
新基準	全体	5207.99±1958.52	6292.61±2446.02	
	16歳以上	5521.06±1610.10	6631.28±2141.61	(P<.001)
	16歳未満	800.19±724.71	1489.00±1042.23	

選択基準変更前後の待機年数および透析年数
（腎単独移植のみ）

旧基準　（1995.4.1 ～ 2002.1.9　N=1,063）
新基準　（2002.1.10～2009.12.31　N=1,327）



レシピエント選択基準変更前後の生存率・生着率

旧基準　（1995.4.1 ～ 2002.1.9　N=1,063）
新基準　（2002.1.10～2009.12.31　N=1,327）

生存率（%）

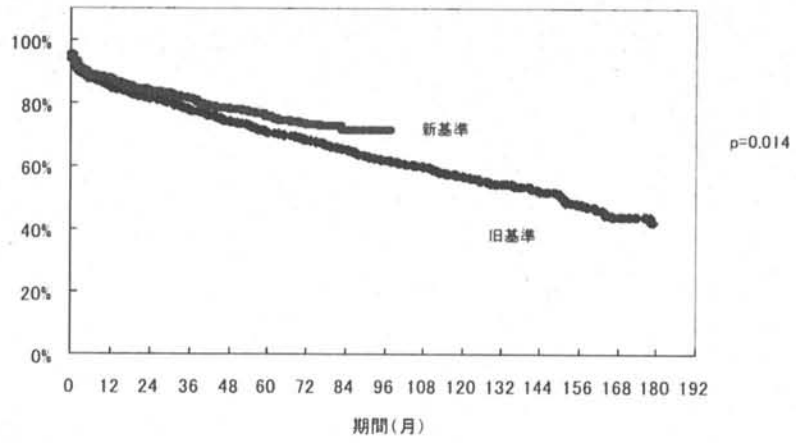
	1カ月	1年	3年	5年	(Logrank)
旧基準	98.2	95.3	91.5	89.3	p=0.135
新基準	98.0	96.1	93.7	91.7	

生着率（%）

	1カ月	1年	3年	5年	(Logrank)
旧基準	91.7	84.9	77.5	70.5	p=0.014
新基準	93.1	87.5	81.2	75.6	

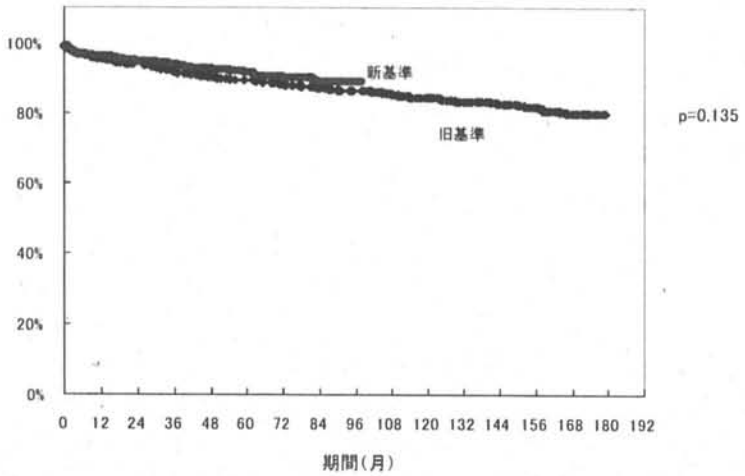
生着率 選択基準別

旧基準　(1995.4.1 ～2002.1.9　N=1,063)
新基準　(2002.1.10～2009.12.31　N=1,327)



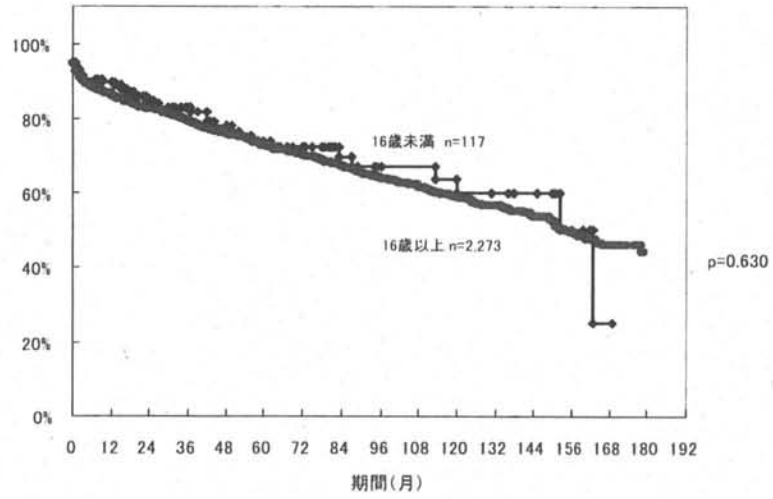
生存率 選択基準別

旧基準　(1995.4.1 ～2002.1.9　N=1,063)
新基準　(2002.1.10～2009.12.31　N=1,327)



生着率　16歳未満・以上

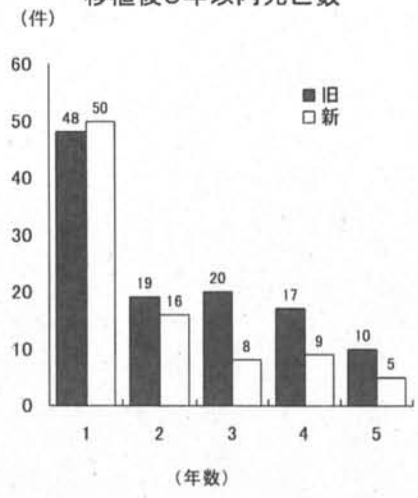
（1995.4.1～2009.12.31）



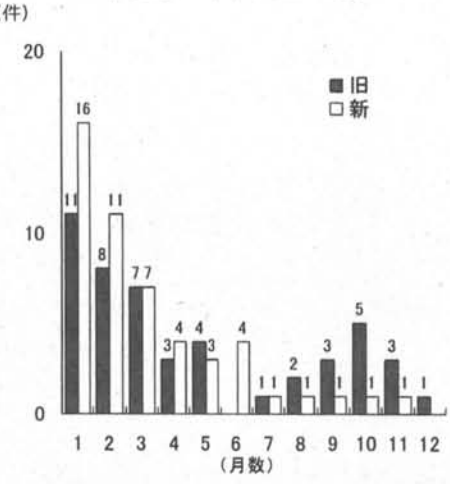
レシピエント選択基準変更前後の移植後死亡数

旧基準　（1995.4.1 ～ 2002.1.9　N=1,063）
新基準　（2002.1.10～2009.12.31　N=1,327）

移植後5年以内死亡数



移植後1年以内死亡数



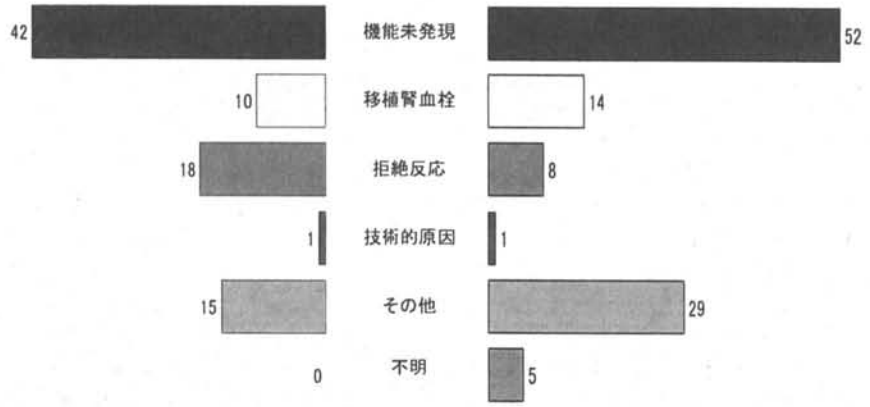
レシピエント選択基準変更前後の移植後無機能腎・術後透析期間・死亡・生着率

旧基準　（1995.4.1 ～ 2002.1.9　N=1,063）
新基準　（2002.1.10～2009.12.31　N=1,327）

	離脱不能 (%)	機能未発現 (%)	術後透析期間 (日)	移植後死亡(%) 3カ月	6カ月	12カ月
旧基準	8.1	4.0	15.07±63.81	2.7	3.2	4.7
新基準	8.2	3.9	12.92±19.71	2.6	3.4	3.8

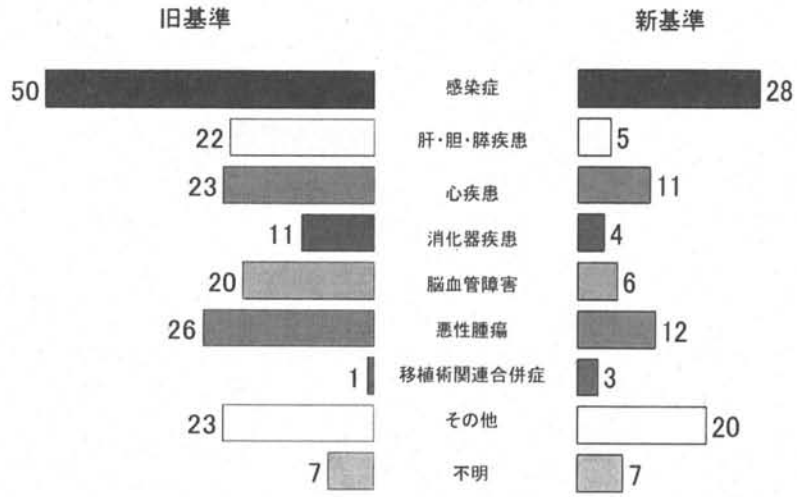
透析離脱不能例とその原因

旧基準		新基準
86/1063 (8.1%)	透析離脱不能例	109/1327(8.2%)
42/1063 (4.0%)	機能未発現	52/1327 (3.9%)

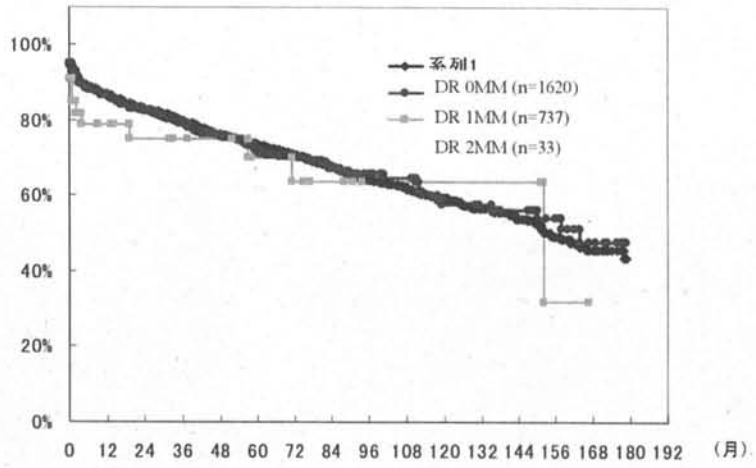


レシピエント選択基準変更前後の移植後死因

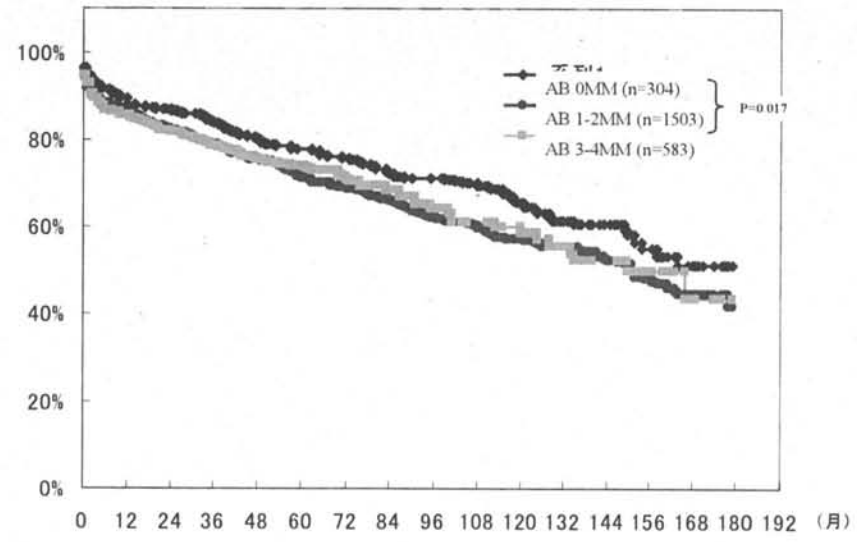
旧基準　（1995.4.1 ～ 2002.1.9　N=1,063）
新基準　（2002.1.10～2009.12.31　N=1,327）



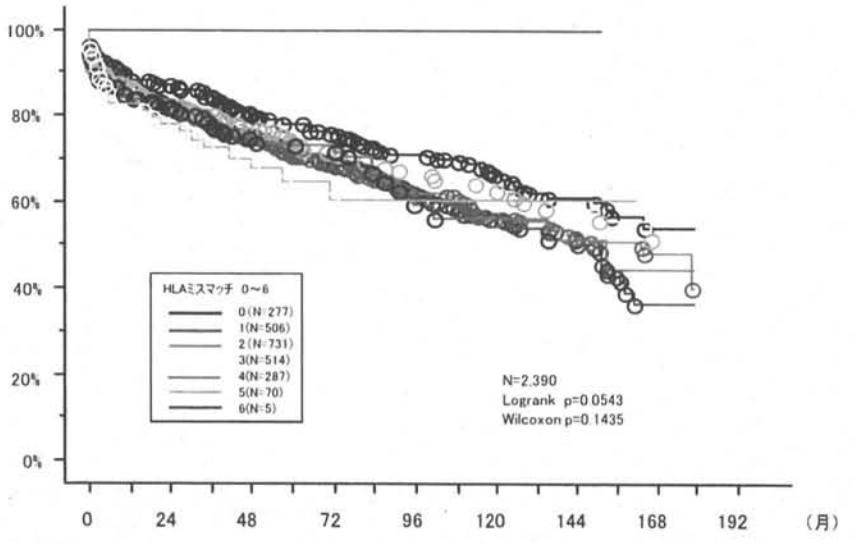
HLA不適合抗原数(検索型DR) 生着率



HLA不適合抗原数(検索型A・B) 生着率



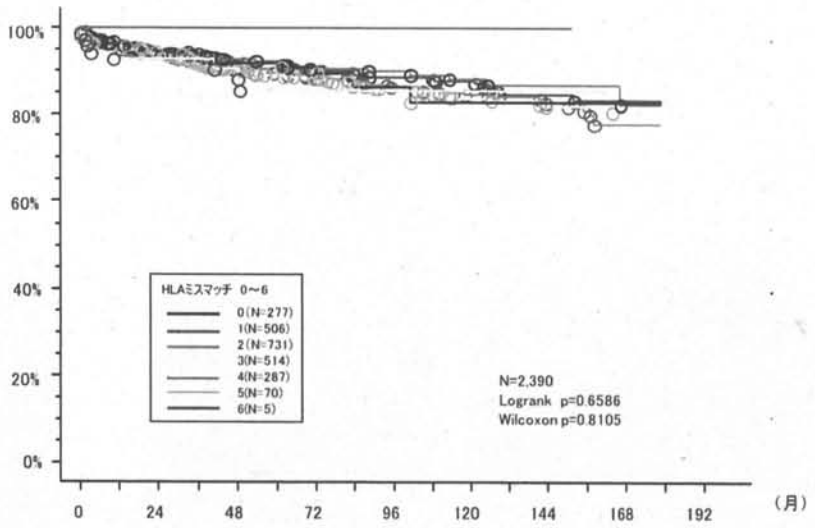
HLA不適合抗原数(検索型A・B・DR) 生着率



HLA不適合抗原数(検索型A・B・DR) 生着率

ミスマッチ数	1年	2年	3年	4年	5年	6年	7年	8年	9年	10年
0	88.4%	87.0%	84.4%	80.2%	77.9%	75.5%	72.3%	71.1%	69.4%	66.3%
1	86.1%	82.3%	78.5%	74.6%	71.5%	68.3%	66.3%	61.6%	58.4%	56.3%
2	86.0%	81.8%	78.6%	75.5%	70.8%	68.9%	65.3%	62.0%	60.5%	56.4%
3	86.9%	82.4%	80.2%	77.4%	74.0%	71.4%	69.7%	67.3%	65.2%	62.7%
4	85.3%	81.6%	77.8%	74.6%	73.9%	71.8%	66.9%	59.6%	55.9%	55.9%
5	82.8%	78.0%	72.5%	67.7%	64.9%	60.3%	60.3%	60.3%	60.3%	60.3%
6	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

HLA不適合抗原数(検索型A・B・DR) 生存率

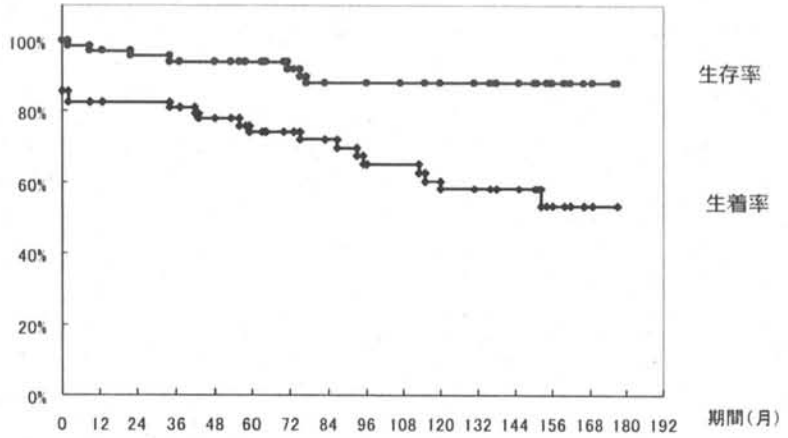


HLA不適合抗原数(検索型A・B・DR) 生存率

ミスマッチ数	1年	2年	3年	4年	5年	6年	7年	8年	9年	10年
0	96.0%	94.9%	94.2%	92.3%	91.5%	89.9%	89.1%	88.7%	88.3%	87.8%
1	95.2%	94.2%	91.7%	90.5%	89.8%	88.4%	87.6%	86.4%	85.5%	84.8%
2	95.7%	93.9%	91.9%	90.4%	89.0%	88.3%	86.7%	85.8%	84.8%	83.7%
3	96.3%	94.5%	93.7%	92.8%	92.4%	90.7%	90.7%	90.0%	89.1%	88.1%
4	96.2%	94.5%	92.3%	91.7%	91.7%	89.7%	86.2%	86.2%	82.8%	82.8%
5	92.8%	92.8%	92.8%	88.0%	85.5%	85.5%	85.5%	85.5%	85.5%	85.5%
6	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

小児提供者(16歳未満)からの(心臓停止後腎臓提供) 腎臓移植 生存・生着

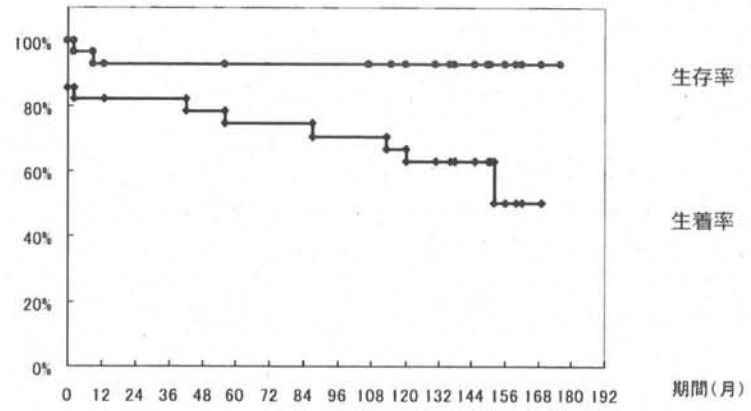
(1995.4-2009.12 N=69)



	1カ月	1年	3年	5年	10年
生存率	100%	98.6%	97.1%	94.0%	87.9%
生着率	85.5%	82.6%	81.1%	74.1%	60.4%

小児提供者(16歳未満)から小児(16歳未満)への(心臓停止後腎臓提供)腎臓移植 生存・生着

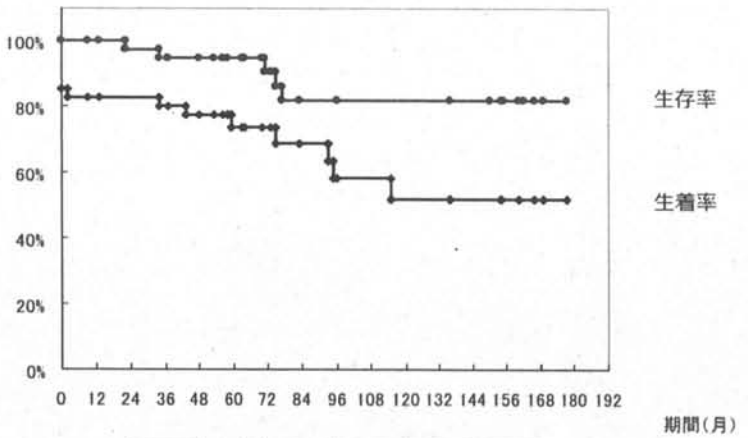
(1995.4-2009.12 N=28)



	1カ月	1年	3年	5年	10年
生存率	100%	92.9%	92.9%	92.9%	92.9%
生着率	85.7%	82.1%	82.1%	74.7%	66.8%

小児提供者(16歳未満)から16歳以上への(心臓停止後腎臓提供)腎臓移植 生存・生着

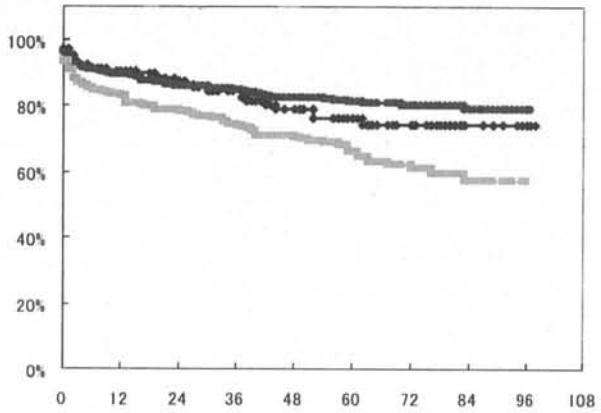
(1995.4-2009.12 N=41)



	1カ月	1年	3年	5年	10年
生存率	100%	100%	94.7%	94.7%	82.0%
生着率	82.9%	82.9%	80.3%	73.7%	51.7%

新基準による腎臓移植　生着率×透析期間

（透析導入日不明のデータを除く）



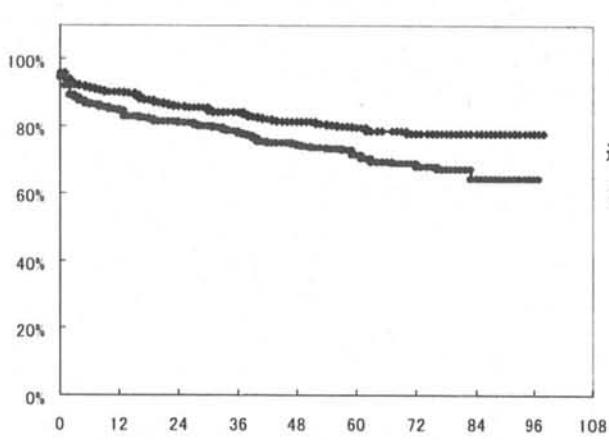
	1カ月	1年	3年	5年
10年未満	95.3%	90.5%	84.5%	76.0%
10年以上	94.1%	89.5%	84.9%	81.2%
20年以上	90.4%	83.0%	74.2%	65.9%

p<0.01

p<0.01

新基準による腎臓移植　生着率×透析期間

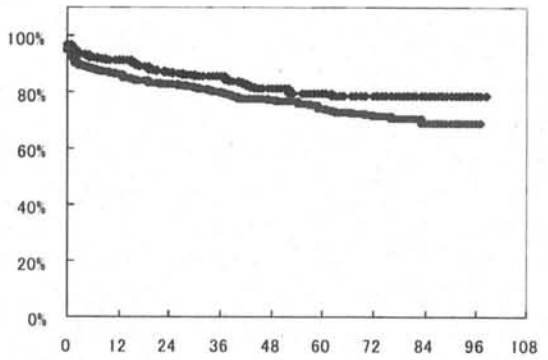
（透析導入日不明のデータを除く）



	1月	1年	3年	5年
17年未満	94.1%	90.1%	84.1%	79.5%
17年以上	91.8%	84.8%	78.4%	71.4%

新基準による腎臓移植　生着率×透析期間

（透析導入日不明のデータを除く）



P=0.02

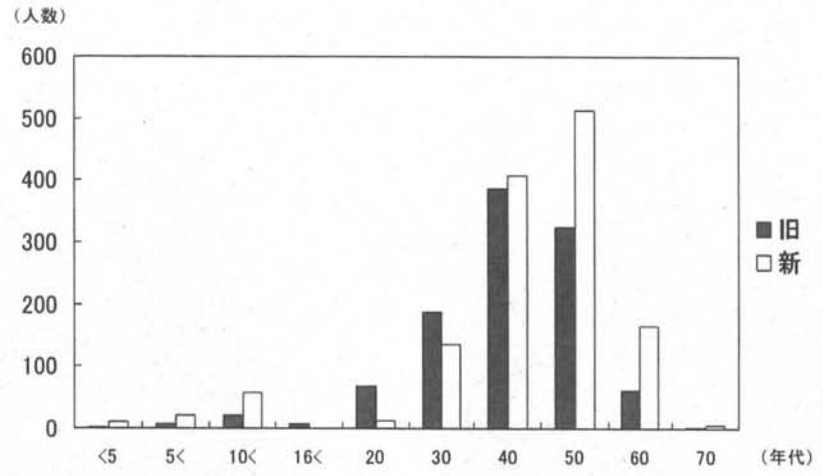
透析13年未満　n=340

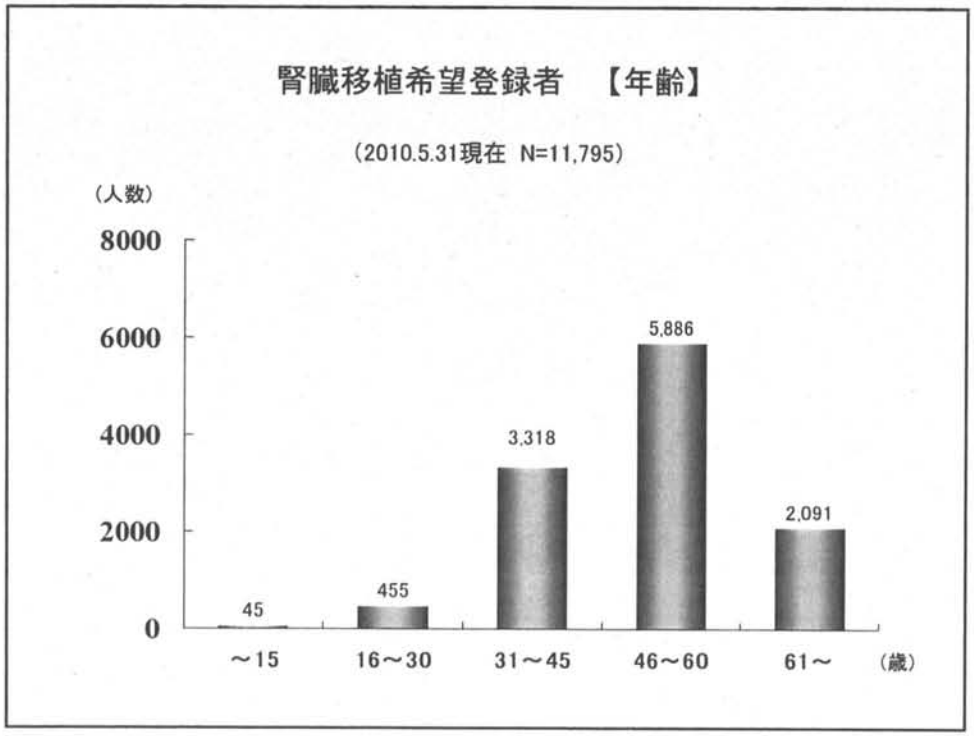
透析13年以上 n=976

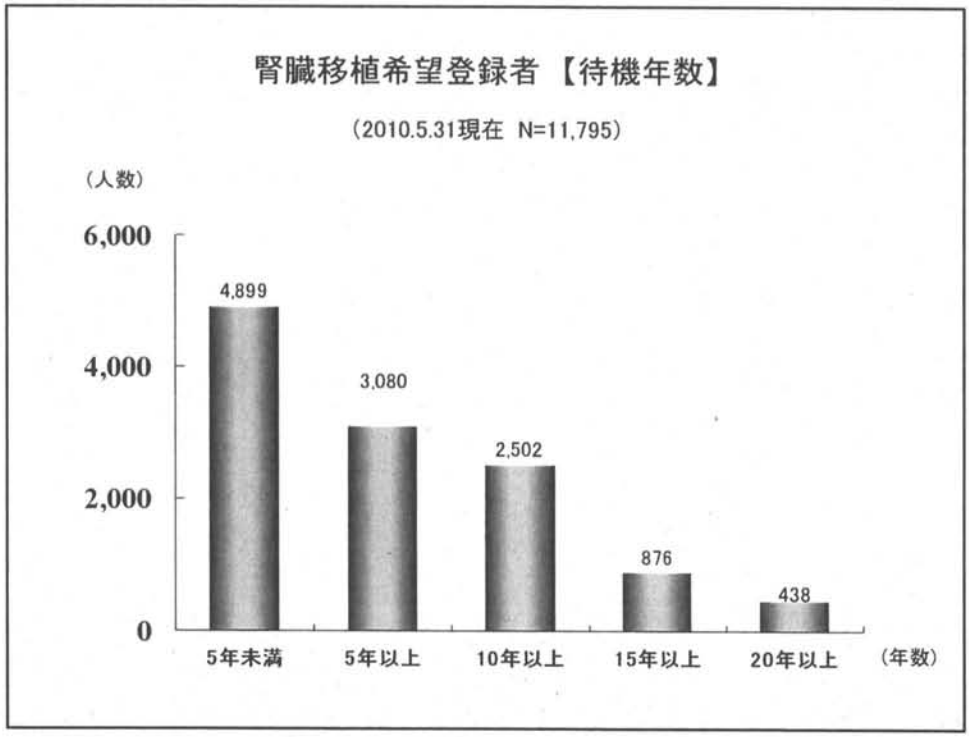
	1月	1年	3年	5年
13年未満	95.3%	91.1%	85.5%	79.8%
13年以上	92.2%	86.1%	79.5%	74.1%

配分基準・年代別移植者数

旧基準　（1995.4.1 ～ 2002.1.9　N=1,063）
新基準　（2002.1.10～2009.12.31　N=1,327）

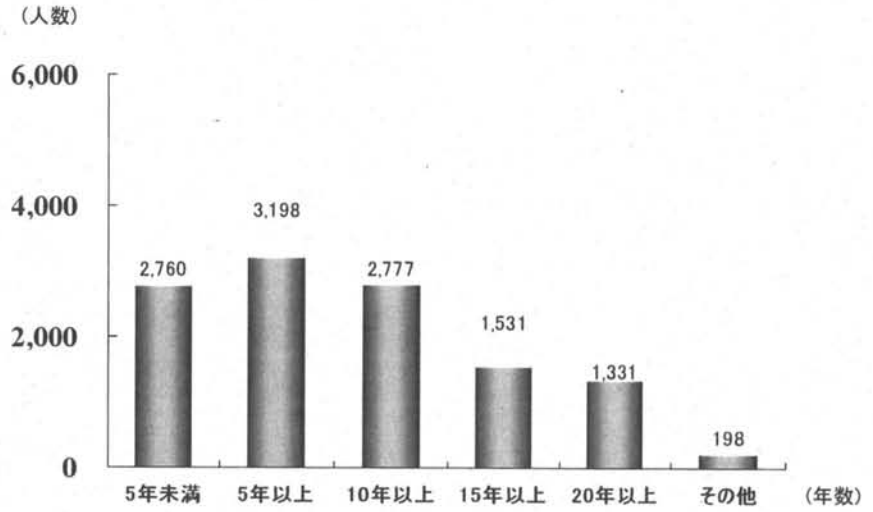



腎臓移植希望登録者　【年齢】
(2010.5.31現在 N=11,795)
(人数)
8000
6000
4000
2000
0
45
455
3,318
5,886
2,091
～15
16～30
31～45
46～60
61～
(歳)


腎臓移植希望登録者 【待機年数】
(2010.5.31現在 N=11,795)
(人数)
6,000
4,000
2,000
0
4,899
3,080
2,502
876
438
5年未満
5年以上
10年以上
15年以上
20年以上
(年数)

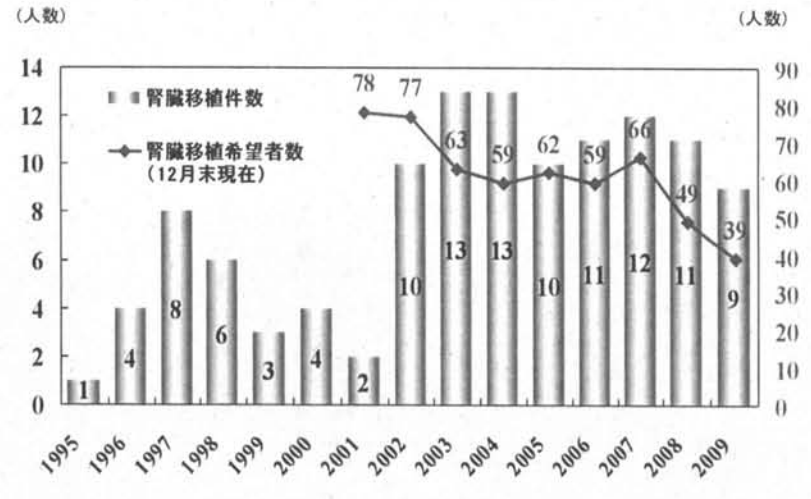
腎臓移植希望登録者【透析年数】

（2010.5.31現在 N=11,795）



小児腎臓移植件数・腎臓移植希望者数の推移

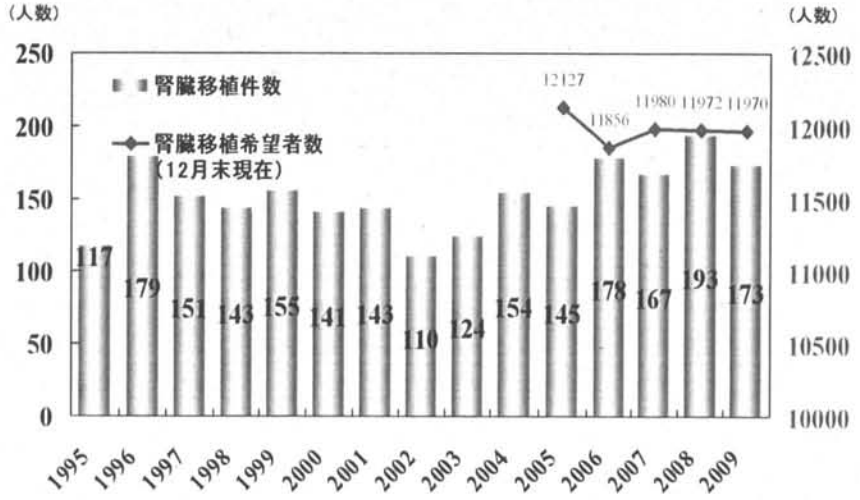
（16歳未満 1995年4月～2009年12月）



＊2002年1月10日より腎臓移植レシピエント選択基準が改正され、小児への移植が優先されるようになった

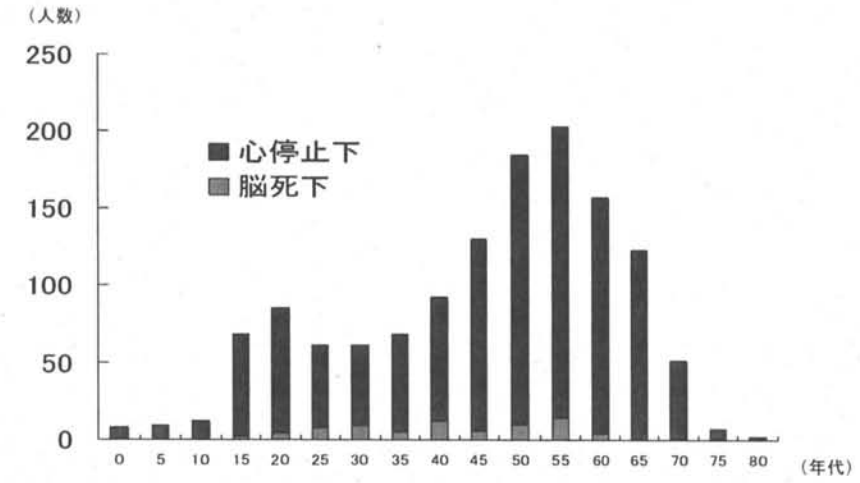
腎臓移植件数・腎臓移植希望者数の推移

（16歳以上　1995年4月～2009年12月）



腎臓提供者【年代】

（1995/4～2009/12　脳死下N=76　心停止下N=1246）



参考資料２

腎臓移植希望者（レシピエント）選択基準

１．前提条件

（１）ＡＢＯ式血液型

ＡＢＯ式血液型の一致（identical）及び適合（compatible）の待機者を候補者とする。

（２）リンパ球直接交叉試験（全リンパ球又はＴリンパ球）陰性

２．優先順位

（１）搬送時間（阻血時間）

地　域	点　数
同一都道府県内（注）	１２点
同一ブロック内	６点

＊　移植希望者の登録地域は移植希望施設の所在地（都道府県）とする。

（２）ＨＬＡの適合度

ＤＲ座の適合数（ミスマッチ数）	Ａ座及びＢ座の適合数（ミスマッチ数）	点　数
0	0	１４点
0	1	１３点
0	2	１２点
0	3	１１点
0	4	１０点
1	0	９点
1	1	８点
1	2	７点
1	3	６点
1	4	５点
2	0	４点
2	1	３点
2	2	２点
2	3	１点
2	4	０点

（3）待機日数

待機日数（N）≦4014 日：待機日数ポイント＝N/365 点

待機日数（N）＞4014 日：待機日数ポイント＝10＋log1.74（N/365－9）点

（4）小児待機患者

小児待機患者（１６歳未満）については１４点を加算する。

3．具体的選択法

適合条件に合致する移植希望者（レシピエント）が複数存在する場合には、優先順位は、以下の順に勘案して決定する。

（1）臓器の移植に関する法律第６条の２の規定に基づき、親族に対し臓器を優先的に提供する意思が表示されていた場合には、当該親族を優先する。

（2）ＡＢＯ式血液型が一致（identical）する者を適合（compatible）する者より優先する。

（3）２．の（1）～（4）の合計点数が高い順とする。ただし、これらの条件が同一の移植希望者（レシピエント）が複数存在した場合には、臓器搬送に要する時間、医学的条件に配慮する。

また、ＰＲＡ検査が可能な場合はＰＲＡ検査陰性を満たすこととする。

（注１）地域は、原則として、都道府県、ブロック内他都道府県とする。ただし、地域の実情を踏まえ、（社）日本臓器移植ネットワークにおいて複数の都道府県を統合したサブブロックを設置することも可能とする。

（注２）１年以内に移植希望者（レシピエント）の登録情報が更新されていることを必要条件とする。

（注３）Ｃ型肝炎抗体陽性ドナーからの移植は、Ｃ型肝炎抗体陽性レシピエントのみを対象とするが、リスクについては十分に説明し承諾を得られた場合にのみ移植可能とする。

（注４）新ルールの下での状況について、実施後１年のデータが蓄積された時点で新ルールを検討するが、必要があれば追加すべき事項について検討する。

参考資料３

＜腎臓＞臓器提供者（ドナー）適応基準

1. 以下の疾患又は状態を伴わないこととする。
   (1) 全身性の活動性感染症
   (2) ＨＩＶ抗体、ＨＴＬＶ－１抗体、ＨＢｓ抗原などが陽性
   (3) クロイツフェルト・ヤコブ病及びその疑い
   (4) 悪性腫瘍（原発性脳腫瘍及び治癒したと考えられるものを除く。）

2. 以下の疾患又は状態が存在する場合は、慎重に適応を決定する。
   (1) 血液生化学、尿所見等による器質的腎疾患の存在
   (2) ＨＣＶ抗体陽性

3. 年齢：７０歳以下が望ましい。

付記　上記の基準は適宜見直されること。

参考資料 4

日本移植学会・日本組織適合性学会　共同作業部会

HLA に関わる選択基準（提言）

（1）現在、腎臓移植の基準等に関する作業班にて「腎移植希望者（レシピエント）選択基準について」改正のため審議中であり、腎移植配分ルールの見直し作業が行われている。 専門領域に携わる日本移植学会、日本組織適合性学会の共同作業部会名にて HLA に関わる選択基準の提言を下記のように行うこととした。

1.　前提条件　（2）リンパ球直接交叉試験（全リンパ球又は T リンパ球）陰性

（修正案）

高感度のリンパ球交叉試験陰性

（解説） 最近は、高感度のリンパ球交叉試験方法が開発されている。とくに Flow cytometry などを用いる方法が該当する。 直接試験とは、もともと交叉試験方法におけるリンパ球に二次抗体を利用しない方法であり、AHG、Flow cytometry は、間接試験と分類される。

3. 具体的選択法 （3） ――また、PRA 検査が可能な場合には、PRA 検査陰性を満たすこととする

（修正案）移植希望者の PRA 検査（HLA 抗体スクリーニング）は、高感度方法を用いて実施することが望ましい。

（解説）PRA 陽性、クロスマッチ陰性は、海外では移植のよい適応となっている。海外では、バーチャルクロスマッチの導入を試みているところもある。候補者の HLA 抗体保有データは、移植レシピエント選択に有用な情報を提供する。

（修正案：下記を追加）

（注5） HLA 検査施設が提供する具体的な検査内容については、関連学会（日本組織適合性学会および日本移植学会）委員会により作成したガイドラインに準拠する。

（2）ドナー発生時における Flow cytometry クロスマッチの緊急対応が可能な施設について話し合った。人員、予算、設備機器不足問題を解決しなければならないが、現在のところ、東北地方では、福島県立医大のみ Flow cytometry が設置されている。東京では、少なくとも 3 施設は必要であると考えられる。

（3）HLA タイピングは、現在、2 桁対応であるが、4 桁は不要である。

Improved Graft Survival in Highly Sensitized Patients Undergoing Renal Transplantation After the Introduction of a Clinically Validated Flow Cytometry Crossmatch

Sandhya Limaye,[1] Patrick O'Kelly,[2] Grainne Harmon,[1] Derek O'Neill,[1] Anthony M. Dorman,[3] Joseph Walshe,[2] John Donohoe,[2] Dilly Little,[4] Peter J. Conlon,[2] and Mary T. Keogan[1,5]

Background. Flow cytometric techniques are increasingly used in pretransplant crossmatching, although there remains debate regarding the clinical significance and predictive value of donor-specific antibodies detected by flow cytometry. At least some of the discrepancies between published studies may arise from differences in cutoffs used and lack of standardization of the test.

Methods. We selected cut-off values for pretransplant flow cytometric crossmatching (FCXM) based on the correlation of retrospective results with the occurrence of antibody-mediated rejection. The impact on long-term renal graft survival of prospective FCXM was determined by comparing graft survival between patients crossmatched with complement-dependent cytotoxicity (CDC) only with those prospectively crossmatched with both CDC and FCXM.

Results. Chosen cut-off values gave a positive predictive value of FCXM for antibody-mediated rejection of 83%, and a negative predictive value of 90%. After the introduction of prospective B- and T-cell crossmatching by flow cytometry in addition to CDC in our center, there was a significant improvement in renal graft survival in highly sensitized patients ($P=0.017$). Four-year graft survival in highly sensitized patients after the introduction of FCXM was 89%, which did not differ significantly from that seen in nonsensitized patients (93%; $P=0.638$).

Conclusions. Our data demonstrate that prospective FCXM improves renal transplant outcome in highly sensitized patients, provided that cut-off values are carefully validated and results interpreted in the context of sensitization history and antibody screening results.

Keywords: Flow cytometry, Crossmatching, Validation, Renal transplant.

(*Transplantation* 2009;87: 1052–1056)

Complement-dependent cytotoxicity (CDC) techniques with or without enhancement are now used in pretransplant crossmatching after Patel and Terasaki's publication in 1969 describing antidonor reactivity in recipient sera (*1*). CDC crossmatching detects preformed, donor-specific, complement-fixing antibodies and was initially introduced to prevent the devastation of hyperacute transplant rejection. However, acute humoral or antibody-mediated rejection (AMR) in the presence of donor-specific alloantibodies is now increasingly recognized as a cause of early renal graft dysfunction with a reported incidence of up to 8% (*2, 3*). As CDC assays do not detect noncomplement-fixing antibodies, nor all complement-fixing antibodies, newer techniques with greater sensitivity are gaining favor and often used in conjunction with CDC.

Flow cytometric crossmatching (FCXM), as first described by Garovoy et al. in 1983 (*4*), has considerably greater sensitivity than the basic or enhanced CDC assays (*4–6, 7*), allows identification of antibody isotype, and detects low-level cytotoxic as well as noncytotoxic antibodies. FXCM techniques are routinely designed to detect IgG donor-specific antibody (DSA). The clinical significance of IgM DSA remains controversial, and IgA DSA is not known to be of clinical significance. Renal transplants performed across a positive flow cytometric crossmatch have been shown to have higher rates of acute rejection, early graft loss, and lower 1-year graft survival (*8*). However, there are conflicting reports in the literature regarding the clinical significance of DSA detected by flow cytometry and not by CDC.

In 2000, 50% of tissue typing laboratories in the United States performed the final pretransplant crossmatch by flow cytometry (*7*), yet the definition of a positive result is not

There are no conflicts of interest for any of the authors involved in the study.

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Received 20 August 2008. Revision requested 6 October 2008.
Accepted 19 November 2008.

ISSN 0041-1337/09/8707-1052
DOI: 10.1097/TP.0b013e31819d17b0

standardized and may account for discrepancies between studies. Positive cut-off definitions from published research studies include more than 2SD shift above mean channel shift from the negative control (*9*, *10*), variable mean channel displacement for T and B cells (*11*, *12*), or more than 3SD increase in sample median fluorescence intensity from negative control values with SD derived from previous crossmatches between nonsensitized sera and donor lymphocytes (*13*). Furthermore, flow cut-off values used to determine a positive or negative crossmatch are not reported as having been validated by retrospective correlation with clinical outcome, with the exception of Kotb et al. (*14*). To date there have been no studies reporting the clinical impact of the introduction of FXCM on graft survival.

We sought to determine optimal flow cytometric cutoff values for B- and T-cell crossmatches by correlating results obtained from crossmatches performed retrospectively with the occurrence of AMR in renal transplant recipients in our center. We also assessed the impact of the introduction of pretransplant crossmatching by flow cytometry using these cutoffs on graft survival.

MATERIALS AND METHODS

All laboratory investigations were performed in the Histocompatibility and Immunogenetics (H&I) laboratory of Beaumont Hospital, which is the national H&I service for solid organ transplantation in Ireland.

All patients diagnosed with AMR between 1998 and 2000 for whom sufficient frozen donor lymphocytes were available for crossmatch studies were included. The clinical outcome of these patients has been previously published (*3*). Control sera from 50 patients receiving renal transplants in the same time period who had not experienced AMR were also included.

The panel-reactive antibodies (PRA) of transplant recipients was assessed on peripheral blood lymphocytes using the National Institute of Health basic CDC technique (*15*) on a selected panel to encompass donor antigens commonly encountered in the Irish population. Potential transplant recipients all received regular screening for anti-human leukocyte antigen (HLA) antibodies by CDC and ELISA (LAT-M/LAT, One Lambda Inc., Canoga Park, CA) every 3 months in line with the European Federation for Immunogenetics standards.

AMR was suspected if there was a clinical evidence of acute graft dysfunction within 6 weeks of transplantation, and confirmed by typical histologic findings of capillary or peritubular polymorphonuclear leukocytes, together with visualization of immunoglobulin or C4 deposits by direct immunofluorescence, or the presence of DSA in patient serum by ELISA or flow cytometry, as per amended Banff criteria (*16*).

Retrospective crossmatches were performed using pretransplant sera of the above renal transplant recipients, all of whom had a negative CDC before transplantation. Flow cytometric crossmatch analysis was performed by modification of a previously described dual-color technique (*17*). In brief, donor cells were incubated with patient or control serum for 30 min at 22°C. Cells were then washed and stained with anti-human IgG conjugated to fluorescein isothiocyanate as well as either anti-CD3 conjugated to phycoerythrin (T-cell stain) or anti-CD19 conjugated to phycoerythrin (B-cell stain) for 30 min at 4°C in the dark. Cells were then washed with FACSFlow, pelleted by microcentrifugation, and finally resuspended in 250 μL FACSFlow. Fluorochrome-conjugated antibodies bound to the cell surface were detected by two-color analysis on a FACScan flow cytometer (BD Biosciences, Franklin Lakes, NJ) and data analyzed with Cellquest software. Results for T- and B-cell crossmatches were expressed as a ratio compared with the corresponding result obtained from male AB serum.

Graft survival was followed up for renal transplants performed at Beaumont Hospital from 1998 to 2005 with information obtained from the National Renal Transplant Registry of Ireland and stratified according to semiquantitative PRA values. To determine the impact of the introduction of prospective FCXM on graft survival, data were divided into two distinct 4-year periods of 1998–2001 and 2002–2005. Renal transplant recipients in the first group all had a negative CDC. Prospective crossmatching by flow cytometry in addition to CDC was introduced into the laboratory in late 2001. Thus, any patients in the second group who had ever had detectable anti-HLA antibodies on screening or who had a sensitizing event within 6 months of transplant received a pretransplant crossmatch by both CDC and flow cytometry.

Immunosuppression regimens given to low-risk patients evolved over the study period from combined cyclosporine and azathioprine, to tacrolimus and azathioprine, and finally, tacrolimus and mycophenolate together with corticosteroids. There was no change in the immunosuppressive regimen given to a subgroup of highly sensitized (PRA ≥50) patients or in surgical or postoperative management between the two time periods. However, from 2002, all positive or equivocal crossmatches were discussed with a consultant immunologist before proceeding to transplantation. Crossmatch results were interpreted in the context of sensitization history and antibody screening results. Transplantation was permitted to proceed with a positive FCXM if the presence of donor-specific anti-HLA antibodies could be excluded.

For statistical analysis, log-rank tests were used to determine differences in graft survival of high PRA patients between the two time periods 1998–2001 and 2002–2005. Cox proportional hazards methods were used in multifactorial models to determine independence of effect of confounding variables on graft outcome. Fisher Exact and Wilcoxon Rank-Sum tests were used to determine differences in demographic and clinical variables. A P value less than 0.05 was considered a significant result. The statistical software used for all analyses was Stata (version 8, College Station, TX).

RESULTS

Twenty patients met criteria for the diagnosis of AMR between 1998 and 2000, of which five were excluded from analysis due to lack of availability of frozen donor cells. Five patients were primary graft recipients and 10 were recipients of second or subsequent renal transplants. Of the 50 selected control sera, one was excluded from analysis due to insufficient cells for accurate analysis, thus a total of 64 retrospective flow cytometric crossmatches for both T and B cells were performed.

Results for T- and B-cell crossmatches were plotted on each axis of a scatter chart with additional identification of the

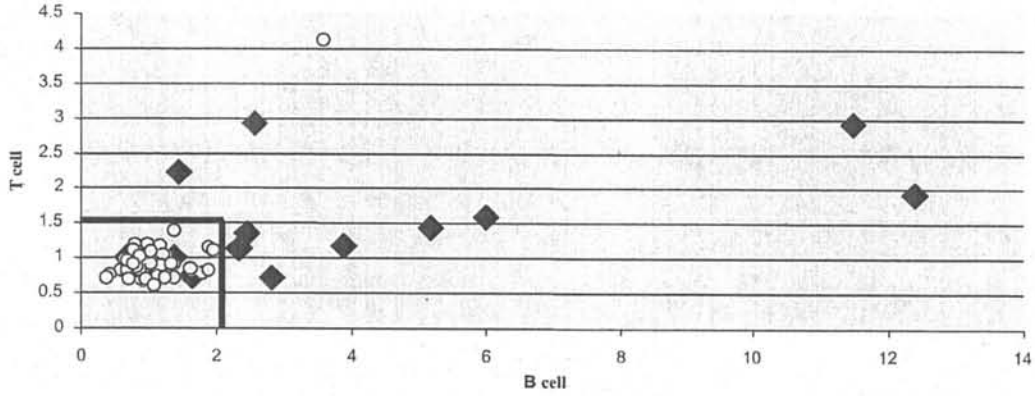

FIGURE 1. Scatter plot of B- and T-cell FCXM results. Patients diagnosed with antibody-mediated rejection are identified with ♦ and controls identified with ○. Cut-off values for a positive crossmatch (T cell >1.5, B cell >2) are delineated and were selected by visual analysis of data distribution.

presence or absence of AMR (Fig. 1). Positive cutoffs were determined by visual analysis of the data and set as a T-cell ratio more than 1.5 and B-cell ratio more than 2. Using these cutoffs, 10 of 15 patients with AMR had a positive crossmatch, giving a sensitivity of 67%. Forty seven of 52 patients with a negative result did not demonstrate features of AMR, giving a negative predictive value of 90%. Of 12 patients with a positive T- or B-cell crossmatch, 10 were diagnosed with AMR, giving a positive predictive value of 83%. Patient 11 was a nontransfused male, whose ELISA screening results were repeatedly negative for anti-HLA antibodies, but had an antilymphocyte antibody detectable by CDC. Hence, appropriate clinical interpretation of results further enhances positive predictive value. Of the 64 patients in this study, only one would have inappropriately denied a transplant because of an FCXM that seemed to be clinically significant.

Graft survival data were obtained from a total of 523 renal graft recipients from 1998 to 2001 and 554 recipients from 2002 to 2005, representing all renal transplants performed within each time period. An increase in graft survival was detected for patients in the latter time period, with a 4-year graft survival of 77% in 1998–2001 compared with 92% in 2002–2005 ($P<0.001$).

When graft recipients in the first time period were stratified into groups of low (≤10%), medium (11%–49%), and high (≥50%) PRA, there were significant differences in graft survival for the different patient groups, with 4-year graft survival of 80%, 75%, and 65%, respectively ($P=0.015$). In contrast, patients who received renal transplants between 2002 and 2005, after the introduction of prospective flow cytometric crossmatching, did not show an inverse correlation between PRA and long-term graft survival, with 4-year survival rates of 93%, 92%, and 89% for low, medium, and high PRA groups, respectively ($P=0.638$) (Fig. 2A). Thus, the introduction of FCXM led to the greatest improvement in 4-year graft survival in patients with high PRA, which increased from 65% to 89%. In addition, there was a significant decrease in biopsy-proven cellular rejection in high PRA patients, from

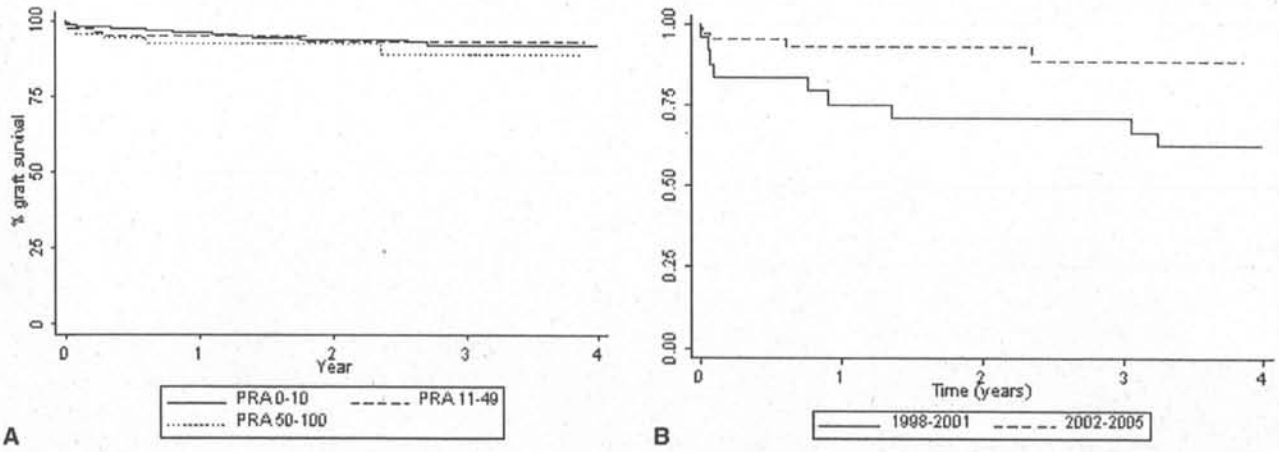

FIGURE 2. (A) Four-year graft survival of renal transplants performed between 2002 and 2005, after the introduction of prospective FCXM. There is no significant difference in graft survival at 4 years in patient groups stratified by PRA. (B) Four-year graft survival of renal transplants in highly sensitized recipients performed in the two time periods before (1998–2001) and after (2002–2005) the introduction of FCXM. All patients received immunosuppression with tacrolimus, mycophenolate, and corticosteroids. Graft survival is significantly higher for patients receiving transplants between 2002 and 2005, after the introduction of prospective FCXM ($P=0.017$).

40.8% in patients receiving transplants in 1998–2001 to 14.9% in those receiving transplants from 2002 to 2005 (P=0.042). The two patient groups with high PRA showed no significant difference in mean donor and recipient age, sex, match grade, length of cold ischemic time nor in the distribution of primary versus regrafts between the two time periods.

A Cox multifactorial regression model was constructed for a number of possible confounders including the above variables as well as posttransplant complications of delayed graft function and biopsy-proven rejection. The change in graft survival between the two time periods remained significant in the presence of these possible confounders (P=0.009). Thus, a reduced risk of graft failure was predicted for the latter time period.

To exclude any change in immunosuppression as a confounder affecting outcome, graft survival between the two time periods was compared between patients with high PRA for whom there was clear documentation of ongoing immunosuppression with tacrolimus, mycophenolate, and corticosteroids.

Figure 2(B) shows that within this group, there was a significant increase in 4-year graft survival from 62.5% between 1998 and 2001 (24 patients) to 88.6% between 2002 and 2005 (64 patients) (P=0.017).

DISCUSSION

Despite a number of studies showing inferior graft outcomes in patients with a positive FCXM, the clinical significance of DSA detected by flow cytometry and not CDC remains controversial. At least one study has shown no difference in the number of rejection episodes or 1-year graft survival among transplant recipients with positive or negative FCXM (*12, 18*). More recently, Vasilescu et al. (*11*) demonstrated that a positive flow crossmatch performed retrospectively was not invariably associated with increased rejection or graft loss. Even studies that have reported increased graft loss in recipients with a positive flow cytometric crossmatch did not demonstrate an increased risk in all patients, raising the possibility of oversensitivity, lack of specificity of a positive result, and the inappropriate exclusion of patients for transplantation. Thus, it is vital that the cutoff values that determine a positive reaction are carefully validated to ensure clinical relevance.

Our data support the well-known increased sensitivity of FCXM compared with CDC crossmatching. Of 64 patients with a negative CDC, 12 patients, or 19% demonstrated a positive T- or B-cell flow cytometric crossmatch. This is consistent with previous reports demonstrating the considerably greater sensitivity of FXCM compared with CDC (*4, 5*). In renal transplant recipients with a negative anti-human globulin (augmented)-CDC, a median of 15% primary grafts and 34% regrafts demonstrate a positive crossmatch by flow cytometry (*8*).

We have shown that the use of cut-off values selected by retrospective correlation of FCXM values with the occurrence of AMR, results in higher positive and negative predictive values than previously reported (*11*). The implementation of these cut-off values in prospective flow cytometric crossmatches in our center resulted in an improvement in long-term graft survival in highly sensitized patients. In agreement with this, it has been reported that graft survival among regraft recipients, a population that usually includes a significant percentage of highly sensitized patients, is significantly increased by prospective crossmatching by flow cytometry (*9*). Because of the evolution in immunosuppression regimens over the study period, the contribution of FCXM to the improvement in graft survival seen in less-sensitized patients cannot be determined.

A prospective study evaluating FCXM showed that transplant candidates with low or negative PRA and a positive flow cytometric crossmatch had significantly greater rates of early rejection and steroid-resistant rejection when compared with FCXM negative controls. Despite this, there was no difference in 1-year graft survival between the two groups. Thus, a prospective positive FCXM in unsensitized patients identified those at increased risk of rejection, although the authors concluded that patients with a positive FCXM should not be excluded from transplantation without consideration of other risk factors such as donor age and degree of sensitization of the recipient (*19*). However, the crucial difference between our study and previous data is the clinical validation of the cutoffs used to determine a positive, equivocal, and negative result.

In our center, the combined intervention of the introduction of prospective, clinically validated FCXM and on-call consultant input has improved graft survival in highly sensitized renal transplant recipients. Since 2001, we routinely performed prospective B- and T-cell flow cytometric crossmatches in patients with any level of detectable anti-HLA antibodies. Unsensitized patients (no anti-HLA antibodies detected by CDC and ELISA or Luminex) do not receive a prospective FCXM; therefore, our findings cannot be extended to this group. Cross-match results are interpreted together with the results of antibody screening, sensitization history, and donor-recipient matching. A positive FCXM attributable to anti-HLA antibodies is a contraindication to transplantation in the absence of augmented immunosuppression. Our data demonstrate that prospective crossmatching by flow cytometry is a useful technique to identify sensitized renal transplant recipients undetected by CDC crossmatching and that the predictive value of the test can be maximized by clinical validation of cutoff values. The decision to proceed with transplantation can be further optimized by interpreting pretransplant cross-match results in the context of the patient's sensitization history and antibody screening results.

ACKNOWLEDGMENT

The authors thank Mr. David P. Hickey, Director of the Transplant Unit for permission to include his patients in the study and for helpful discussions.

REFERENCES

1. Patel R, Terasaki PI. Significance of the positive crossmatch test in kidney transplantation. *N Engl J Med* 1969; 280: 735.
2. Crespo M, Pascual M, Tolkoff-Rublin N, et al. Acute humoral rejection in renal allograft recipients. I. Incidence, serology and clinical characteristics. *Transplantation* 2001; 71: 652.
3. Abraham KA, Brown C, Conlon PJ, et al. Plasmapheresis as rescue therapy in accelerated acute humoral rejection. *J Clin Apher* 2003; 18: 103.
4. Garovoy MR, Rheinschmidt MA, Bigos M, et al. Flow cytometry analysis: A high technology crossmatch technique facilitating transplantation. *Transplant Proc* 1983; 15: 1939.

5. Chapman JR, Deierhoi MH, Carter NP, et al. Analysis of flow cytometry and cytotoxicity crossmatches in renal transplantation. *Transplant Proc* 1985; 17: 2480.
6. Leenaerts PL, De Ruysscher D, Vandeputte M, et al. Measurement of alloantibody by flow cytometry. *J Immunol Methods* 1990; 130: 73.
7. Gebel HM, Bray RA, Nickerson P. Pre-transplant assessment of donor-reactive, HLA-specific antibodies in renal transplantation: Contraindication vs. risk. *Am J Transplant* 2003; 3: 1488.
8. Christiaans MHL, Overhof R, ten Haft A, et al. No advantage of flow cytometry crossmatch over complement-dependent cytotoxicity in immunologically well-documented renal allograft recipients. *Transplantation* 1996; 62: 1341.
9. Vasilescu ER, Ho EK, Colovai AI, et al. Alloantibodies and the outcome of cadaver kidney allografts. *Hum Immunol* 2006; 67: 597.
10. Gebel HM, Bray RA. Sensitisation and sensitivity: Defining the unsensitized patient. *Transplantation* 2000; 69: 1370.
11. Bryan CF, Baier KA, Nelson PW, et al. Long-term graft survival is improved in cadaveric renal retransplantation by flow cytometric crossmatching. *Transplantation* 1998; 66: 1827.
12. Nelson PW, Eschliman P, Shield CF, et al. Improved graft survival in cadaveric renal retransplantation by flow crossmatching. *Arch Surg* 1996; 131: 599.
13. Michelon T, Schroeder R, Fagundes I, et al. Clinical relevance of low levels of preformed alloantibodies detected by flow cytometry in the first year post-kidney transplantation. *Transplant Proc* 2005; 37: 2750.
14. Karpinski M, Rush D, Jeffery J, et al. Flow cytometric crossmatching in primary renal transplant recipients with a negative anti-human globulin enhanced cytotoxicity crossmatch. *J Am Soc Nephrol* 2001; 12: 2807.
15. Kotb M, Russell WC, Hathaway DK, et al. The use of positive B cell flow cytometry crossmatch in predicting rejection among renal transplant recipients. *Clin Transplant* 1999; 13(1 Pt 2): 83.
16. Scornik JC, Clapp W, Patoon PR, et al. Outcome of kidney transplants in patients known to be flow cytometry crossmatch positive. *Transplantation* 2001; 71: 1098.
17. Racusen LC, Colvin RB, Solez K, et al. Antibody-mediated rejection criteria—an addition to the Banff '97 classification of renal allograft rejection. *Am J Transplantation* 2003; 3: 708.
18. Bray RA, Lebeck LK, Gebel HM. The flow cytometric cross-match: Dual color analysis of T and B cell reactivities. *Transplantation* 1989; 48: 834.
19. Hopkins KA. The basic lymphocyte microcytotoxicity tests: Standard and AHG enhancement. In: Hahn AB, Land GA, eds. Laboratory Manual. American Society for Histocompatibility and Immunogenetics 2000, pp IC 1.1.

ELSEVIER

Use of Kidneys From Anti-HCV Positive Donors

J.M. Morales, J.M. Campistol, A. Andres, B. Dominguez-Gil, N. Esforzado, F. Oppenheimer, and J.L. Rodicio

PEREIRA et al[1] demonstrated that transmission of HCV infection occurred in 100% of the patients receiving a kidney from an HCV RNA positive donor and 50% of infected patients developed chronic liver disease. This observation in the early 1990s has led several procurement centers to advocate that all HCV-infected kidneys should be discarded. This policy has remained controversial because there are other studies showing that transmission of HCV infection and the subsequent development of chronic liver disease were uncommon.[2] The heterogenicity of data on the rate of transmission has not been well explained, but undoubtedly renal transplant patients who receive kidneys from HCV-positive donors have a high risk on liver disease.[1] Therefore, according to current information the widely held opinion is that HCV-positive kidneys should not be transplanted into HCV-negative recipients.

The problem is that a complete restrictive policy of discarding kidneys from HCV-positive donors will aggravate organ shortage. Some authors have therefore suggested that kidneys from HCV-positive donors should be transplanted into HCV-positive recipients.[3] There are several arguments against this approach and solid arguments for the use of these kidneys, mainly that organ shortage is very important and many patients on the waiting list for transplantation will die before receiving a kidney transplant.

In 1990, our two hospitals in Spain introduced the policy of accepting kidneys from HCV-positive donors for HCV-positive recipients after full information and informed consent.[4] The Spanish Transplant Organization supported this policy. The results of our prospective study showed no differences in terms of the prevalence of liver disease (32% vs 56%, respectively), graft survival (96% vs 93%) and patient survival (100% vs 98%) in 24 anti-HCV positive patients who received kidneys from anti-HCV positive donors compared with 40 anti-HCV positive recipients who received kidneys from HCV negative donors.[5] These results are in agree with retrospective studies from Los Angeles[6] and Washington[7] and indicate that transplantation of kidneys from anti-HCV positive donors into HCV-positive recipients is relatively safe at least for a period of 5 years.

However, in our study, when HCV RNA was determined in all available serum, we showed that 80% of HCV-positive but RNA-negative patients who received a kidney from an HCV RNA-positive donor turned to have a positive HCV RNA after transplantation, 50% of them developing chronic liver disease.[5] Therefore, these results suggest that HCV-positive kidneys should be offered to HCV RNA-positive recipients only. So, this policy was adopted in our Hospitals in 1993. In HCV-positive patients in our waiting list, HCV RNA is determined every 6 months. Widell et al[8] pointed out that superinfection is possible with a new genotype[8] although in a short-term follow up there were no important clinical consequences. Matching donors and recipients for HCV genotype would be desirable to minimise the risk of superinfection. HCV-positive patients on the waiting list for transplantation should be therefore tested for HCV RNA and genotype.

In Spain, kidneys from HCV-positive donors are transplanted in some hospitals into HCV-positive recipients only. Long-term experience (10 years) with this policy shows that liver disease (50% vs 44%), graft (74% vs 79%), and patient survival (89% vs 87%) are not different in 98 patients compared to 158 HCV-positive recipients who received organs from HCV negative donors.[9]

In summary, because of shortage of organs, we propose that HCV-positive donors may be offered to HCV RNA-positive recipients. Full information and informed consent are mandatory. Matching donors and recipients for HCV genotype would be desirable and measures to minimize the effect of HCV infection in renal transplant patients should be recommended.

REFERENCES

1. Pereira BJG, Milford EL, Kirckman RL et al: N Engl J Med 327:910, 1992

2. Morales JM, Campistol JM, Andres A, et al: Curr Opin Nehrol Hypertens 7:201, 1998

3. Diethelm AG, Roth D, Fergusson RM, et al: N Engl J Med 326:410, 1992

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0041-1345/01/$–see front matter
PII S0041-1345(00)02675-0

4. Morales JM, Andres A, Campistol JM: N Engl J Med 328:511, 1993

5. Morales JM, Campistol JM, Castellano G, et al: Kidney Int 47:236, 1995

6. Mendez R, Aswad S, Bogaard T, et al: Transplant Proc 25:1487, 1993

7. Ali MK, Light JA, Barhyte DY, et al: Transplantation 66: 1694, 1998

8. Widell A, Mansson S, Persson NH, et al: Transplantation 60:642, 1995

9. Morales JM, Campistol JM: J Am Soc Nephrol 11:1343, 2000

第3回腎臓移植の基準等に関する作業班

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場　所：厚生労働省　共用第8会議室