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Graft and Patient Survival after Primary Cadaver Kidney Transplantation

with special regard to compatibility in the HL-A system

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BENGT STORM

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Chapter 1

INTRODUCTION

Transplantation of ''non-self'' tissue is inevitably followed by rejection of the transplanted tissue in the unmodified individual unless complete genetical identity exists between donor and recipient. The first successful kidney transplantation in man in 1954 was based on genetical identity between donor and transplant (Merrill et al., 1956). A successful transplantation of kidney between genetically non-identical but closely related individuals was first achieved in 1959 after total body irradiation of the recipient (Hamburger et al., 1959). After the development of new immunosuppressive agents, especially the 6-mercaptopurine-derivative azathioprine (Calne, 1961, Starzl et al., 1963) kidney transplantation with organs from living related donors became a therapeutic method during the middle of the sixties. The success with related donors stimulated the application of the method also in non-related living and cadaveric donors. The great advantages in the use of non-related donors were however offset by poorer results, apparently dependent upon increasing immunological differences between donor and recipient.

The immunological differences between individuals of the same species were first studied in mice. Several antigen systems of varying importance for transplantation, histocompatibility antigen systems, were identified. One of the systems named the H-2 system was found to be of dominating importance for graft acceptance and rejection (Snell, 1968). Transplantation of skin grafts with incompatible H-2-antigens resulted in shorter graft survival than transplantation of grafts with other incompatible antigens (H-1 to H-11). Similar major histocompatibility systems were found in other species, Ag-B in rat, H-1 in rabbit, Locus B in chicken etc.

In man the ABO-system was found to be a major histocompatibility system. Transplantation of a kidney to a recipient with A or B antibodies was ''safe'' only when the kidney came from a donor without A or B antigen (Starzl et al., 1964).

Dausset found in 1954 that multiple blood transfusions to a patient could give an immunological response by formation of humoral antibodies directed against the donor leukocytes. Antibodies against leukocytes were also demonstrated to be formed after pregnancy (van Rood et al., 1958) and after experimental skin grafting (Walford et al., 1962). The antigens, giving rise to leukocyte antibodies as an immune response of the host after transfusions, pregnancies or transplantations were found to constitute a complex system, first called the Hu-1 system, later the HL-A system (*H*uman *L*eukocyte, locus *A*) (Dausset et al., 1965a. Dausset et al., 1967, Bach & Amos, 1967). In many ways this system was found to be homologous with the H-2 system of the mouse (Davies et al., 1968). Like the A and B antigens in the ABO-system HL-A antigens were found not only on leukocytes but also on platelets and cells from various tissues in man. HL-A antigens are now thought to be present on cells from most of the normal human tissues (Berah et al., 1970). The nomenclature, the characteristics, the methods of determina-

tion and other details of the HL-A system are described elsewhere (Dausset, 1971 a and b, Kissmeyer-Nielsen & Thorsby, 1970, Thorsby et al., 1971b).

The universally present HL-A antigens may by definition give rise to humoral leukocyte antibodies in a recipient after transplantation of tissue cells with a non-identical HL-A specificity. This was found to occur occasionally after blood transfusions as well as after transplantation of skin and other organs (Morris et al., 1969b). It was also found that these leukocyte antibodies present in a recipient after previous sensitization impaired the survival of the transplanted organ (Friedman et al., 1961, Dausset et al., 1965 a and b, Kissmeyer-Nielsen et al., 1966, Gallinaro et al., 1967, Morris et al., 1968b). Was the *possibility* of formation of these HL-A-antibodies of any importance for the graft survival, and what did the immunosuppressive therapy, necessary in clinical organ transplantation, mean?

Skin graft survival was found to be prolonged when HL-A identity existed between sibling donor and recipient (Ceppellini et al., 1969, Amos et al., 1969). An increasing degree of HL-A incompatibility was found to decrease the survival time of skin grafts from living related donors when no immunosuppression was given (Dausset et al., 1970). Some authors found a certain but less convincing correlation when unrelated skin donors were used (Ceppellini et al., 1969, Batchelor & Hacket, 1970).

Renal graft survival and function was found to be superior with HL-A identical sibling donors compared to non identical sibling donors (Singal et al., 1969, Starzl et al., 1970, Hors et al., 1971, Mickey et al., 1971). Regarding other living related donors most authors have found better results with better HL-A compatibility (Rapaport et al., 1967, Lee et al., 1967, Terasaki, 1968, Hume et al., 1969, Kissmeyer-Nielsen et al., 1969, van Rood et al., 1969, Singal et al., 1969, Kissmeyer-Nielsen et al., 1970 and 1971, Hamburger et al., 1971, Dausset & Hors, 1971, Perkins et al., 1971) while some authors found uncertain or no correlation (Starzl et al., 1971, Hors et al., 1971, Mickey et al., 1971, Halgrimson et al., 1971).

Concerning transplantations with renal grafts from unrelated cadaveric donors many authors found better results with HL-A identical donors. Some investigators found however no difference in the results if there existed one or more HL-A incompatibilities (Morris et al., 1969a, Payne et al., 1971, Festenstein et al., 1971a, Hamburger, 1971), while others found better results with lower incompatibility grades (Batchelor et al., 1971, Dausset & Hors, 1971, Festenstein et al., 1971b, Kissmeyer-Nielsen et al., 1971, Perkins et al., 1971). There were also several reports from extensive clinical series with no correlation between histocompatibility grades and clinical result after renal transplantation with either related or unrelated donors (Hume et al., 1969, Halgrimson et al., 1971, Mickey et al., 1971, Sheil et al., 1971, Starzl et al., 1971).

Opinions as to the importance of the HL-A system for the results of renal transplantation are thus very divergent. Except for the deleterious effect of preformed HL-A antibodies against donor lymphocytes there is unanimity of opinion only regarding the superiority of transplantation with HL-A identical siblings. However, this superiority has not been proved to be due to the HL-A identity; other genetic factors may be responsible in this very special situation. It may also be stated from a review of the literature that the consensus of opinion is that transplantations with grafts from HL-A identical unrelated donors do better than transplantations with grafts from HL-A non-identical unrelated donors. Any other conclusion cannot be drawn regarding the influence of the HL-A system on the results of renal transplantation. Theoretically however, the HL-A system would be expected to have greater influence on the results of kidney transplantations, particularly since the transplantation antigens in other species have been found to be of profound influence for the outcome of organ transplanta-

tion within these species. The lack of correlation between the results of clinical organ transplantation and the tissue compatibility within the HL-A system may be due to imperfect or incorrect 1) selection of patient materials, 2) HL-A typing methods, and/or 3) interpretation of results.

Ad 1. Transplantation, especially with nec-rokidneys, is a very complex therapeutic method and the clinical results are influenced by numerous factors. The method is used on very different patient categories (with different diseases, ages, degrees of uremia etc.) and the grafts are of very different "quality" and handled in different ways. Even more important the treatment, especially the immunosuppressive therapy, varies considerably. One of the great difficulties is to obtain a patient material large enough but yet reasonably uniform and uniformly treated to be able to study just one variable factor, the HL-A compatibility. In many published reports the results from several transplantation centres have been pooled to increase the number of patients in the series. This has had the disadvantage of increasing the heterogeneity of the patient material and patient treatment. It has also increased the difficulties in evaluating uniformly the transplantation results obtained and assessed in different transplantation centres. The efforts spent in obtaining large patient series have thus led to such an increasing heterogeneity of patient material, patient treatment and clinical evaluation that conclusions regarding the influence of the HL-A system no longer can be drawn.

Ad 2. The accuracy of the HL-A typing method has improved during the last few years and has also varied from centre to centre. This means that variously incomplete HL-A typed transplantations have been compared to and grouped with more accurately HL-A typed transplantations. To be able to evaluate the importance of the HL-A system it is necessary to characterize the HL-A antigens accurately and to use a uniform, standardized method of HL-A antigen identification. This demand for accuracy and uniformity of the method of HL-A typing has often been disregarded.

Ad 3. The results of clinical transplantations are usually expressed in graft survival curves. Graft survival time and graft loss are easily and objectively determined but do not depend on one single factor. Should all graft losses be registered or some excluded as due to other factors than those being examined? In most of the published reports some of the graft losses have been excluded. The reason for these exclusions has been based more on opinions than on facts. The interpretation of the clinical results has therefore been too uncritical.

Chapter 2

PURPOSE

The purpose of this study was to investigate the importance of HL-A compatibility and incompatibility for graft and patient survival during the first year after primary necrokidney transplantation, great care being taken to avoid the above-mentioned three sources of errors.

The recipients were selected for transplantation at *one* centre on equal conditions, transplanted and cared for on *one* ward with standardized immunosuppression and other treatment. The whole patient material was known personally by the author who participated in the treatment and follow-up of every patient in this series. The grafts were preserved by a standardized method. The degree of HL-A compatibility was determined retrospectively according to the actual knowledge of the HL-A system. The transplantation results were registered without any exclusions.

Chapter 3

MATERIAL

Preoperative tissue-typing according to the HL-A system was introduced in the Department of Surgery I, Sahlgren's Hospital, Göteborg in November 1968. During the first months the typing technique was so uncertain that not even a retrospective evaluation of the typing protocols made a determination of the compatibility grade between donor and recipient possible. From February 1969 the typing technique was standardized sufficiently to allow the degree of HL-A compatibility between donor and recipient to be determined by retrospective reevaluation of the typing protocols according to the actual knowledge of the HL-A specificities. Transplantations performed from February 1st 1969 were therefore included in this study. After November 5th 1970 new graft preservation techniques and new immunosuppressive regimes were introduced which made critical comparison with earlier transplantations impossible. Transplantations performed after November 5th 1970 were therefore considered unsuitable and were not included in this study. During the period February 1st 1969 to November 4th 1970 95 consecutive primary necrokidney transplantations were performed.

When evaluating the results of kidney transplantation it is of great importance to avoid unwarranted exclusions of patients from the material. It is also essential to have a uniform standardized method of patient treatment. This is especially true regarding such treatment which might interfere with the factors under investigation. Since the purpose of this study was to evaluate the significance of the degree of HL-A compatibility, a factor of possible immunological importance, it was essential to have a standardized immunosuppressive therapy. Some recipients were given an immunosuppression different from the routine regime. Therefore 17 transplantations were excluded from this study; these were eight patients given extra-corporeal irradiation of the blood preoperatively and nine patients treated postoperatively with antithymocyte or antilymphocyte globulin. This therapy was given as a trial and the patients were chosen exclusively dependent on the actual facilities to give extra-corporeal irradiation of the blood, antithymocyte or antilymphocyte globulin.

For the same reasons nine transplantations were excluded in which the grafts were treated with continuous plasma perfusion according to Belzer (Belzer & Kountz, 1970). Continuous plasma perfusion might affect immunological reactions in different ways. Plasma containing humoral antibodies can give rise experimentally to hyperacute rejection in the recipient (Collste et al., 1970) and clinical experience indicates that an immunological interference of plasma exists. Continuous perfusion decreases the number of so called passenger leukocytes and preserves the viability of the graft better which might influence the antigenicity of the kidney (Stuart et al., 1971 a and b). The continuous perfusion method was chosen to obtain clinical experience in nine cases in which the other kidney from the same donor was also used for transplantation after traditional preservation; no other selection was made.

Four grafts were removed at the time of

Diagnosis	Patients	
	No	Per cent
Pyelonephritis	27	42
Glomerulonephritis	23	36
Polycystic kidney disease	11	17
Eclampsia with cortical necrosis	1	5
Malignant hypertension	1	
Hypernephroma in single kidney	1	

Tab. I. Primary causes of uremia.

transplantation because of technical failures (thrombosis, bleeding). One kidney was hyperacutely rejected and removed at the same operation. In this case the obligatory preoperative cross-match had been considered negative but reexamination showed a weak positive reaction. The hyperacute rejection in this case was undoubtedly due to the presence of preformed humoral antibodies. These five transplantations in which the grafts were immediately removed were also excluded.

This study thus comprised 64 primary necrokidney transplantations on 64 patients with a uniform treatment of both the grafts and the recipients.

Recipients

Since the transplantation program started in Göteborg in 1965 the aim has been to give every patient with terminal uremia the chance of transplantation. The indications have therefore been liberal and the contraindications few. Patients in this series were accepted up to the age of 60. Absolute contraindications were malignancy (except kidney tumours), diabetes requiring treatment with insulin, active tuberculosis, severe psychic disorders and severe coronary arteriosclerosis. The recipients came from all parts of Sweden. Up to the time of transplantation they were cared for in their home districts or in the regional nephrological department. The patients were generally not called to Göteborg until the transplantation was forthcoming.

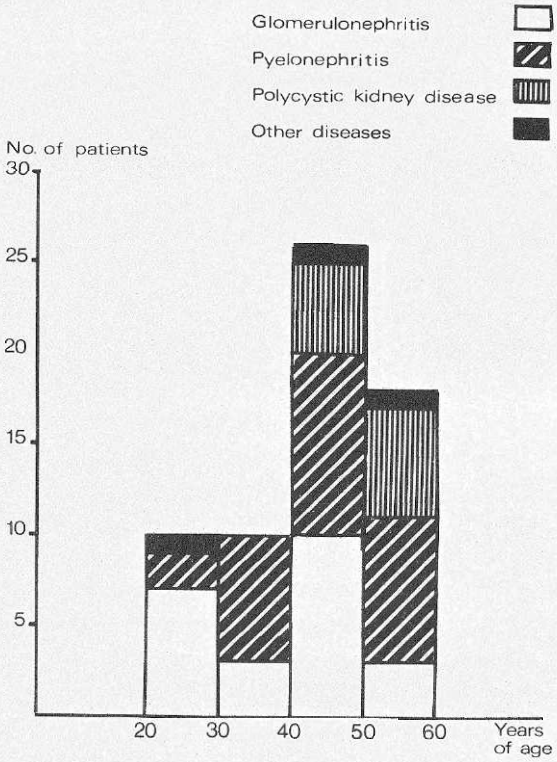

Fig. 1. Age distribution and cause of uremia of the recipients.

Age, sex, cause and stage of uremia

All the diagnoses of the primary renal diseases of the patients were confirmed histologically after biopsy or after nephrectomy. In the case of a non-specific history or non-specific pathology a reexamination of the histological sections was made and the diagnosis settled after thorough discussion. The cause of uremia was chronic pyelonephritis in 27 patients (42%), chronic glomerulonephritis in 23 patients (36%) and polycystic kidney disease in 11 patients (17%). Three patients with uremia caused by eclampsia with cortical necrosis, malignant hypertension with nephrosclerosis and hypernephroma in a single kidney were also transplanted (Table I).

The age range of the recipients was 22 to 59 years (Fig. 1). Twenty patients (31%) were younger than 40 years. 45 patients (70%) were males with a mean age of 47 years, 19 were females with a mean age of 41 years.

Though heterogeneous, the pyelonephritic

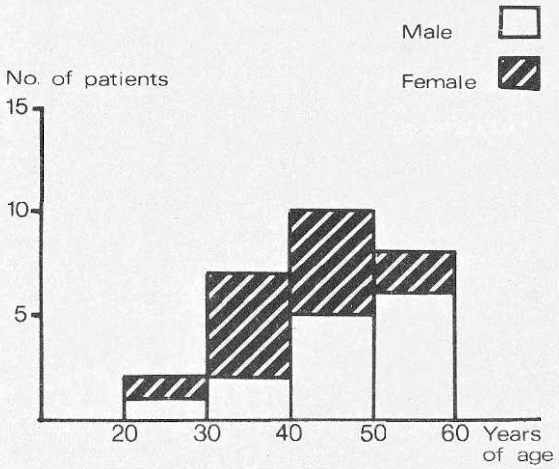

Fig. 2. Age and sex distribution of patients with pyelonephritis.

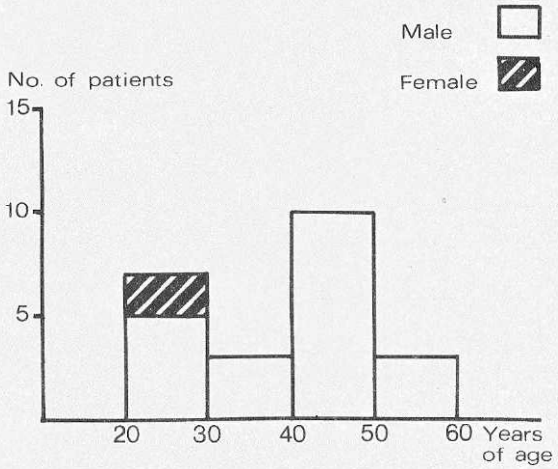

Fig. 3. Age and sex distribution of patients with glomerulonephritis.

group was discussed as a unit. The diagnosis pyelonephritis was used because of international practice although a more adequate designation would have been interstitial nephritis as the diagnosis pyelonephritis included patients with obstructive features, congenital abnormalities and drug abuse. About half of the patients admitted abuse of phenacetin-containing drugs. The highest frequency of the disease was in females in the age group 30–50 years, while in males the incidence increased with increasing age (Fig. 2).

The 23 patients with glomerulonephritis were also discussed as one group. Various kinds of glomerulonephritis, possible immunological activity, duration or progress tempo of the disease etc, were not considered. There were predominantly males in this group (Fig. 3).

The eleven patients with polycystic kidney disease were transplanted at the age of about 50.

The patients in this series did not differ from the total material of primary necrokidney transplantations during the same period with regard to age, sex or cause of uremia (Table II and III).

	No	Sex distribution		Age distribution			
		Male	Female	Male		Female	
		Per cent	Per cent	Mean	Range	Mean	Range
All primary necrokidney recipients	95	67	33	46	22–59	39	19–55
Investigated material	64	70	30	47	22–59	41	24–54

Tab. II. Age and sex distribution in the series of patients investigated and in the series of all 95 patients.

	No	Pyelonephritis Per cent	Glomerulonephritis Per cent	Polycystic kidney disease Per cent	Other diseases Per cent
All primary necrokidney transplantations	95	39	42	14	5
Investigated material	64	42	36	17	5

Tab. III. Distribution of primary renal diseases in the series of patients investigated and in the series of all 95 patients.

Different kidney diseases lead to terminal uremia at various rates. To be able to estimate the rate of progress of each patient the aim of our department was to be informed about patients with incipient uremia as early as possible. Some patients in this study therefore were waiting for the transplantation for shorter or longer time in a fairly good condition. Other patients had a rapid progress into uremia or did not come for examination until they had reached a final uremic state. The facilities for hemodialysing during the actual time also varied very much from place to place and from time to time. Thus the degree of uremia in the recipients, their general condition and the frequency of uremic complications varied considerably. In this study the duration of preoperative dialysis was accounted for, while such uncertain factors as mentioned above were not considered. In this material 12 patients (19%) were transplanted in a predialytic phase, 16 patients (25%) after dialysis up to 3 months, 29 patients (45%) after dialysis for 3–12 months and 7 patients after more than 12 months of dialysis. The average waiting time for transplantation was 6 months with wide variations.

Nephrectomy of recipient kidneys

The importance of removal of the patients' own kidneys prior to transplantation is disputed and doubtful. Both renal infections and renal hypertension might jeopardize the transplantation results. Hume and others hold the opinion that the risk of recurrent glomerulonephritis in the graft is greater if the patients' own kidneys are left in place (Hume et al., 1970). Because of these possible disadvantages of the patients' own kidneys being left in place our indications for nephrectomy before transplantation during 1969 and 1970 were:

1. Rapidly progressing glomerulonephritis.
2. Hypertension in patients on hemodialysis.
3. Recurrent urinary infections in patients on hemodialysis.
4. Urinary output of less than 300 ml/day in patients on hemodialysis.

As many patients were treated at other hospitals before the transplantation these rules were not followed regularly.

Altogether 15 patients were nephrectomized prior to transplantation (Table IV).

Nephrectomy at the time of transplantation (always on the ipsilateral side) was performed in five cases with single kidneys or with urinary infections. 44 patients had their own kidneys left at the time of transplantation; 12 of them were nephrectomized within two months after the transplantation nine of them within one year. The general condition of the patient, the degree of hypertension and frequency of urinary infections determined the most suitable time for nephrectomy. It is obvious that patient selection existed with regard to nephrectomy before transplantation as well as after transplantation. There was no mortality in connection with these nephrectomies.

Donors

Patients dying in hospitals or brought in to hospitals immediately after death were used as donors of the grafts in this series. Excluded from donation were persons over 70 years of

	No.	Own kidneys removed prior to transplantation	Own kidneys removed at transplantation	Own kidneys left at transplantation
Pyelonephritis	27	3	4	20
Glomerulonephritis	23	10	1	12
Total investigated material	64	15	5	44

Tab. IV. Time for nephrectomy of own kidneys (last kidney) in investigated series.

age, persons with known or noted kidney disease, hypertension or malignancy except for tumours in the central nervous system. Only in five cases was donor nephrectomy performed while the donor was on artificial respiration. Cardiac arrest was awaited in the remaining cases. Males dominated as donors, 45 (70%) were males (Fig. 4). Trauma with or without head injury was the cause of death in 33 cases (52%), subarachnoid hemorrhage in 17 cases (27%) (Table V). One third of the donor kidneys came from smaller neighbour hospitals, one third from other transplantation centres in Scandinavia and one third from our own hospital. To preserve organ viability bloodless hypothermia was induced immediately after the donor nephrectomy. The preservation technique used was the method described by Brunius et al (1968) in all but two kidneys with very short warm ischemia times which were perfused with "Collins' solution" (Collins et al., 1969).

The premortem period of the donor was often protracted with failing circulation, asphyxia, disturbances in fluid- and electrolyte balance, failing renal function with oliguria or anuria, hypothermia etc. Some of these factors might have had great significance not only for the viability but also for the immunogenicity of the kidney. In this study special attention was given to the length of the warm ischemia time (i.e. the time from cardiac arrest until the kidney was perfused and cooled) (Fig. 5) and the cold ischemia time (i.e. the total time from the initial perfusion until the reperfusion in the recipient) (Fig. 6). Grafts with a warm ischemia time of more than 60 minutes were not accepted. 19 grafts (30%) had a warm ischemia time shorter than 20 minutes. The cold ischemia time varied from 160 to 1000 minutes, but most of the grafts, 46 (72%) had a cold ischemia time of 5–11 hours.

Diagnosis of the donor	No. of kidneys used
Head injury	27
Subarachnoid hemorrhage	17
Extensive trauma	6
Intoxication	5
Cerebral tumor	4
Myocardial infarction	3
Other	2

Tab. V. Diagnoses of the donors of the grafts.

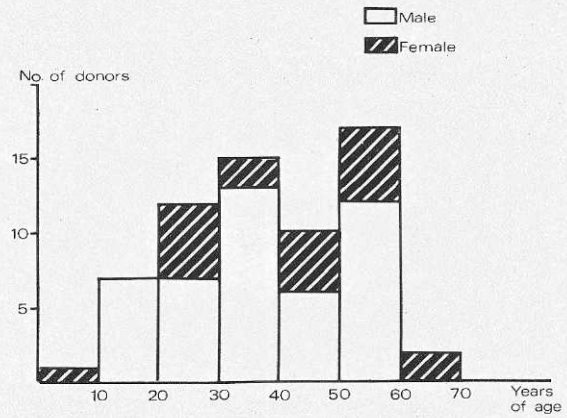

Fig. 4. Age and sex distribution of the donors.

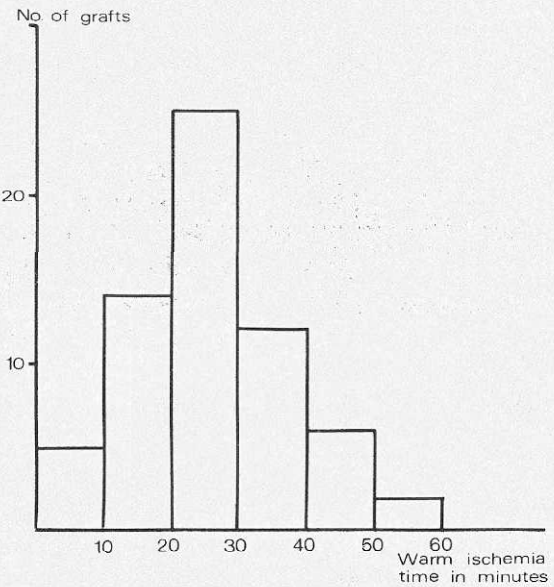

Fig. 5. Duration of warm ischemia.

The degree of ischemic damage of the kidney was to some extent reflected in the length of the postoperative anuric or oliguric period. The "day of onset" of renal function was registered as the first day with spontaneously decreasing serum creatinine. Six grafts (9%) started to function on the day of operation, ten (16%) the day after while 19 (30%) started two weeks or more after the transplantation (Fig. 7).

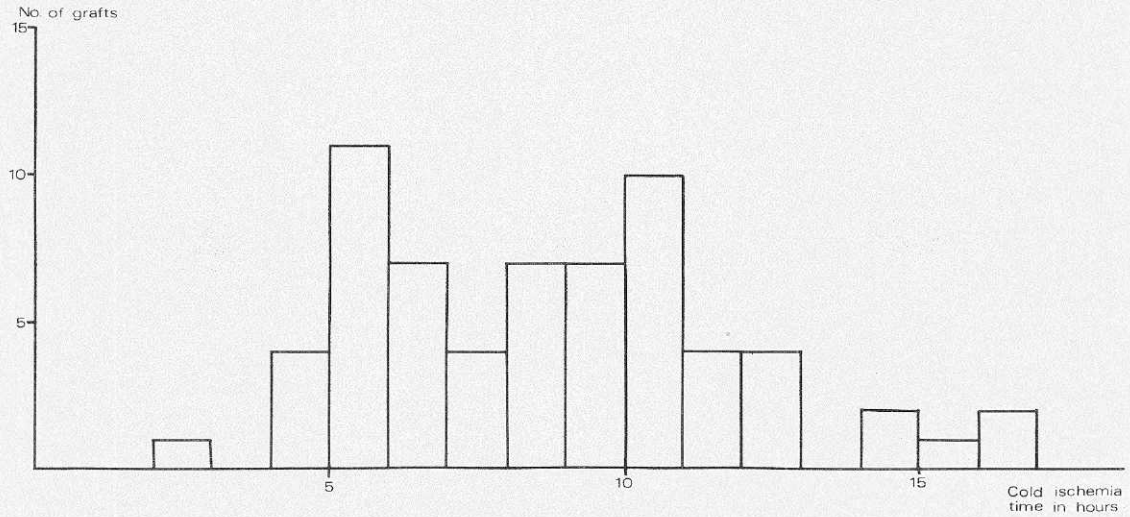

Fig. 6. Duration of cold ischemia.

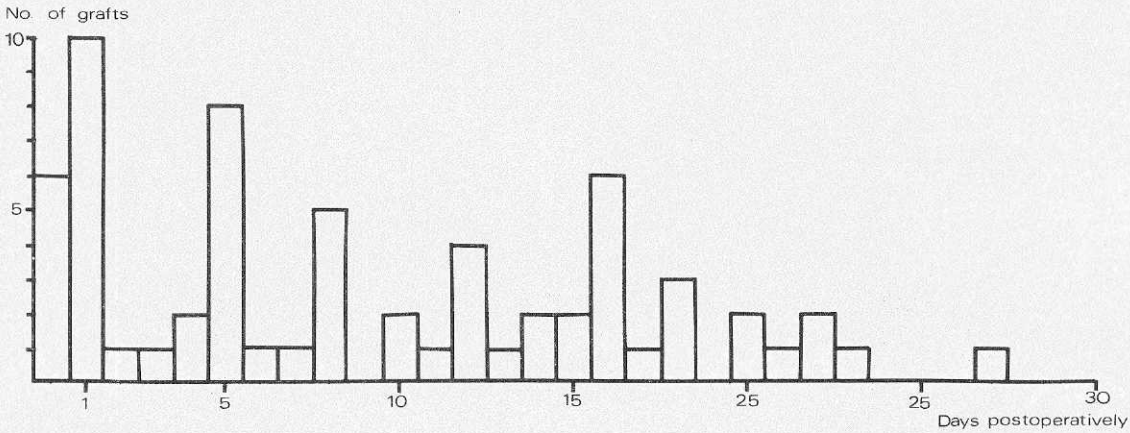

Fig. 7. Day of onset of renal function.

Chapter 4

METHODS

Clinical method

Every available necrokidney was offered to bloodgroup compatible recipients within the Scandiatransplant organization, comprising all transplantation centres in Sweden, Norway, Denmark and Finland and including also the city of Hamburg, with a common registration of all recipients. Priority was given to recipients to whom an available kidney was HL-A compatible over recipients to whom the graft was incompatible in one HL-A antigen. Grafts with one proved incompatibility were generally accepted independent of the possibility of further unknown antigens, except for patients in a very good condition and still in a predialytic phase of uremia when a compatible graft was pursued. No grafts with two or more known HL-A incompatibilities were accepted for patients in this series and no patients were transplanted as a desperate therapeutic attempt.

Every transplantation was preceded by a direct cross-match of donor lymphocytes and a fresh sample of recipient serum to identify preformed humoral HL-A antibodies against the donor lymphocytes. The transplantation operations were carried out as acute operations as soon as graft and recipient were prepared.

The operative technique, the postoperative care as well as the immunosuppressive therapy was well standardized in this series of transplantations. An experienced transplantation surgeon was responsible for every transplantation. The kidney was placed extraperitoneally with the renal artery anastomosed end-to-end to the internal iliac artery and the renal vein end-to-side to the external iliac vein. End-to-side anastomosis to the external iliac artery was used in a few cases. Two or more renal arteries were anastomosed if necessary. The donor ureter was implanted in the bottom of the bladder. A ureteral catheter was used as a splint to assure proper urine outflow. The catheter was removed when a normal serum creatinine was established or after 10-14 days. Splenectomy or thymectomy was not performed.

The patients were treated postoperatively on a special ward by a well trained nursing staff. The author participated in the care and follow-up of every patient in this series.

Postoperative dialysis treatment was preferably given as peritoneal dialysis for the first 4–6 days to avoid local bleedings. Hemodialysis was then resumed if required.

No prophylactic antibiotics were given but manifest infections were treated with appropriate antibiotics.

Prophylaxis against gastric ulcer was given with antacidic and vagus blocking drugs.

Prophylaxis against oral fungal infections was given by local applications of methylrosaniline, nystatine and sodium bicarbonate. Adequate antihypertensive drugs, cardiotropic or other necessary drugs were used individually on special indications. Diuretics were not used routinely.

As thrombosis prophylaxis and to improve the blood flow in the graft early in eventual rejection episodes low molecular weight dextran (Rheomacrodex®), was given routinely the first three postoperative days and then twice weekly during the hospital stay.

Diagnosis of rejection

When decreasing kidney function or other clinical signs of rejection such as fever, pulmonary symptoms, enlargement and tenderness of the graft etc. led to the suspicion of rejection, other prerenal, renal or postrenal causes were first dismissed before the diagnosis of rejection was made. Routinely a renal angiography was performed on the 4th–6th postoperative day. In case of suspected rejection another renal angiogram was made to exclude other diagnoses and to strengthen the diagnosis of rejection (Nilsson et al., 1968). If there was any doubt about the diagnosis of rejection a needle biopsy was performed. A rejection episode was defined as reversible as long as graft function was maintained and irreversible when graft function failed. Every non-functioning graft in this series was removed.

Immunosuppressive therapy

The immunosuppressive therapy was based on prednisolone and azathioprine.

Prednisolone was given preoperatively in a dose of 100 mg and postoperative initially 200 mg/day.then in a successively decreasing dose to about 1 mg/day/kg body weight a fortnight after the transplantation. The dose was then further reduced to 10–20 mg/day 2–3 months after the transplantation. The mean daily dose to recipients with remaining grafts was 1.3±0.0 mg/kg during the first postoperative month and the mean maintenance dose one month after the transplantation 0.7±0.0 mg/kg.

Azathioprine was given preoperatively as early as possible (usually only some hours before operation) in a dose of 100 mg and postoperatively in a dose of 3 mg/kg body weight/day as long as no side effects were registered. Because of side effects, especially leukopenia, the azathioprine dose often had to be lowered. In fact this intended dose was given to only 31 of the 54 recipients with grafts remaining for at least one month. The mean daily dose during the first postoperative month

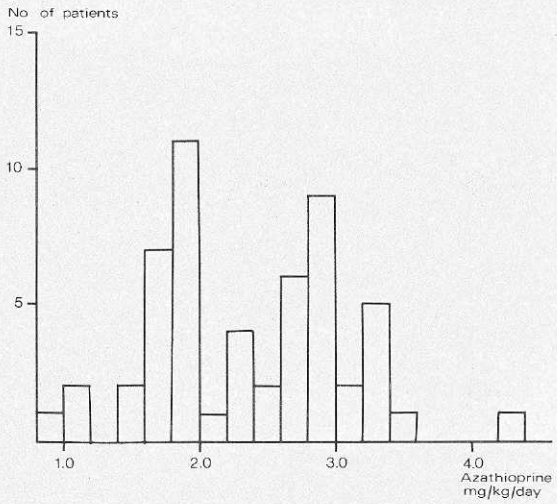

Fig. 8. Mean daily azathioprine dose given during the first postoperative month.

for these 31 patients was 2.9±0.1 mg/kg. The other 23 patients received a mean daily dose of 1.7±0.1 mg/kg (Fig. 8).

No other immunosuppressive therapy was given routinely in this series. When an acute rejection was diagnosed the prednisolone dose was increased for one or two days, actinomycin C was added for five days (200 gamma every day) and local x-ray irradiation to the graft was given in a dose of 150 rad every second day three or four times. The azathioprine dose was maintained.

Long-term control

The postoperative hospitalization time varied from three weeks to three months. The patients were then controlled at their local hospitals as out-patients one to four times a month. Thorough follow-up examinations on the transplantation ward were made 6 and 12 months after the operation and included estimations of inulin- and PAH-clearance, protein excretion and renal angiography.

All removed grafts were examined histopathologicallly and special care was taken in cases where recurrent glomerulonephritis could be suspected.

All patient who died were autopsied.

Statistical methods

The data in this study are presented as graft survival curves and patient survival curves. The graft and patient survival time was calculated for every transplantation and expressed as "whole months after the day of transplantation" up to 12 months. All kinds of patient and graft losses were registered without any selection. Patient loss e.g. also meant graft loss even if the graft functioned well at the time of death of the recipient. The percentage of graft and patient survivals was calculated for every postoperative month. Averages were calculated and expressed as arithmetic means with the standard error of the mean.

Frequency differences between groups were calculated according to the χ^2-method (K. Pearson) and using the tables of Fisher's Statistical Method for Research (Oliver & Boyd, Edinburgh and London, 1936).

Methods for histocompatibility determination

Together with A. Lindholm

Tissue-typing technique

The blood group antigens within the ABO-system and the antigens according to the HL-A system were considered. The HL-A antigens were determined by the lymphocytotoxicity microtechnique described by Terasaki & Mc Clelland (1964) and modified by Kissmeyer-Nielsen & Kjerbye (1967). The lymphocyte suspensions were prepared in two different ways, one with the albumin flotation technique (Kissmeyer-Nielsen & Kjerbye, 1967) and another with the technique described by Böyum (1968) and modified by Thorsby & Bratlie (1970). The HL-A antigens of the donors were determined with these two lymphocyte suspensions separately. The lym-

	HL-A antigens	Identified by antisera no.	At the time of
1st series 2nd series	1, 2, 3, 9, 11, 28 7, 8, 12, 13, W5, W10, W15	29	February 1969
1st series 2nd series	1, 2, 3, 9, 11, 28, 10 7, 8, 12, 13, W5, W10, W15, 5, 27, W16, W22, 5(AJ), W15(AJ), 27(AJ), W22(AJ)	45	December 1969
1st series 2nd series	1, 2, 3, 9, 11, 28, 10, W19 7, 8, 12, 13, W5, W10, W15, 5, 27, W16, W22, 5(AJ), W15(AJ), 27(AJ), W22(AJ), 14, W18(SL), W21(SL), 17	63	October 1970

Tab. VI. HL-A antigens identified during the investigation period.

phocytotoxic reactions were read by a trained serologist.

During the period of this investigation the number of anti-sera used and thereby the accuracy of the HL-A antigen identification increased. The increasing number of test sera was due to a continuous screening and selection of sera obtained from women with multiple pregnancies, patients receiving multiple blood transfusions and from transplanted patients. The exchange of test sera within the Scandiatransplant organization also implied an improvement in the quality of the test sera. The lymphocytes from the very first donors and recipients in this material were tested against 29 anti-sera but already during 1969 the number of anti-sera increased to 45. These anti-sera identified seven antigens of the first series and 15 of the second series. The number of anti-sera increased successively to 63, identifying eight antigens in the first series and 19 in the second series (Table VI).

The specificities of some anti-sera were not known at the time of testing; later experience has identified them. The protocols from all donor and recipient typings were therefore reevaluated according to the actual knowledge of the anti-sera used and their specificities. The HL-A type of every donor and recipient was then settled. This retrospective reevaluation increased the accuracy and uniformity of the determination of the HL-A-antigens.

Evaluation of compatibility grade

In 24 of the recipients and 38 of the donors only two or three of the four theoretically possible antigens were identified at the time of transplantation. This may be due to either the occurrence of homozygotism of one or two antigens or to the presence of unknown HL-A antigens. The evaluation of the compatibility grade in such cases has been under debate. Some authors exclude all "non-full-house" transplantations because of the possible uncertainty in the determined HL-A phenotype. The accuracy of a determined HL-A phenotype depends however not only on the number of identified antigens but also on the antibody titer and specificity of each anti-serum used. No exclusions of "non-full-house" identifications were made in this study.

To be able to account for the possibility of further non-identified HL-A antigens in case of "non-full-house" identification, different methods have been used earlier. Some have prefered to calculate every possible incompatibility and use the term "worst possible compatibility grade" for the evaluation (Kissmeyer-Nielsen et al., 1970). Others have calculated the ratio between found and possible incompatibilities and used this ratio to determine the compatibility grade (Rapaport & Dausset, 1970). None of these methods consider however the other possible explanation for the non-identification of one or two antigens, namely the possibility of homozygotism. Therefore none of these methods was accepted for this study. The compatibility grade was instead determined in the following ways:

1. Independent of the number of possible incompatibilities only the number of *proved* incompatibilities was considered.
2. a)
   When different reactions against anti-sera(s) existed with the donors's and the recipient's lymphocytes this was regarded as a sign of different HL-A combinations even if the incompatible antigen was not identified. In this way the *most probable* compatibility grade was determined in five "non-full-house" transplantations.

   b)
   When no different reactions existed and only two or three antigens were identified the chance for homozygotism was calculated and the risk of further incompatibilities was considered. According to known gene-frequencies of different HL-A antigens among a population of 690 unrelated individuals, tested in Göteborg, the chance for homozygotism was calculated (Sandberg & Lindholm) (Table VII).

If the gene frequency of one single defined antigen in one locus was "a" and the frequency of the sum of the unknown antigens in the actual series was "u" the chance for homozygotism was $\frac{a}{a+2\mu}\,^{100}$ per cent. Because of the varying number of anti-sera used during the investigation period the "0"-gene-frequency varied. In every homozygotism calculation the actual "0"-gene-frequency at that time was used (i.e. the given figures of chances for homozygotism in Table VII were not always used).

	HL-A antigen	Gene-frequency	Chance for homozygotism if only one antigen is identified in the locus Per cent
First series (LA-locus)	1	0.1462	90.9
	2	0.3277	95.7
	3	0.2013	93.2
	9	0.0895	86.0
	10	0.0393	72.9
	11	0.0545	78 9
	28	0.0545	78.9
	W19	0.0797	84.5
	"0"	0.0073	
Second series (4 locus)	5	0.0356	22.6
	7	0.1687	58.0
	8	0.1236	50.3
	12	0.1259	50.7
	13	0.0166	12.0
	W5	0.0646	34.6
	W10	0.1129	48.0
	W15	0.1033	45.8
	14	0.0228	15.7
	W22	0.0116	8.7
	27	0.0476	28.0
	W21(SL)	0.0095	7.2
	17	0.0263	17.7
	W18(SL)	0.0207	14.5
	W16	0.0095	7.2
	5(AJ)	0.0065	5.1
	W15(AJ)	0.0060	4.7
	27(AJ)	0.0207	14.5
	W22(AJ)	0.0065	5.1
	"0"	0.0611	

Tab. VII. Frequencies of identified genes among 690 investigated, non-related individuals and chance of homozygotism if only one antigen has been defined in the actual locus.

If the calculated chance for homozygotism exceeded 50% no further antigens were accounted for, if the chance for homozygotism was less than 50% one more antigen, non-identified, was presumed. After this calculation the *most probable* compatibility grade was determined.

According to these calculations the results of the transplantations in this study were correlated to:

1) *the number of* ***proved*** *incompatibilities,*

2) *the number of* ***most probable*** *incompatibilities.*

When no HL-A incompatibility was proved or probable, HL-A compatibility was said to exist.

At the time of transplantation no incompatibility was found on 26 occasions, one incompatibility on 38 occasions. Reevaluation of the typing protocols revealed one further transplantation with one proved incompatibility and two with two incompatibilities.

When different serological reactions and the chances for homozygotism were considered no incompatibility most probably existed in 14 transplantations, one incompatibility in 36 and two incompatibilities in 14 transplantations (Fig. 9).

Limitations of the method of compatibility determination

HL-A typing technique

The technique of HL-A identification is an accurate method though involving several evaluations of the strength of cytotoxic reactions with considerations of both the viability of the lymphocytes and the various strengths and specificities of numerous anti-sera. Because of the great number of anti-sera used for every HL-A identification false positive or negative reactions could hardly be overlooked without coming to the attention of a trained serologist.

Only two subloci in the HL-A system were considered. The possibilities of other loci have been discussed but not yet proven and were therefore not considered in this study (Thorsby et al., 1970).

Compatibility determination

The degree of compatibility must be considered somewhat uncertain even in "full-house" transplantation. The "0"-antigen frequencies are now fairly small but further splitting of the already known antigens into more "narrow", specific antigens can be expected (Thorsby et al., 1971b). This will always tend towards a lowering of the compatibility grade.

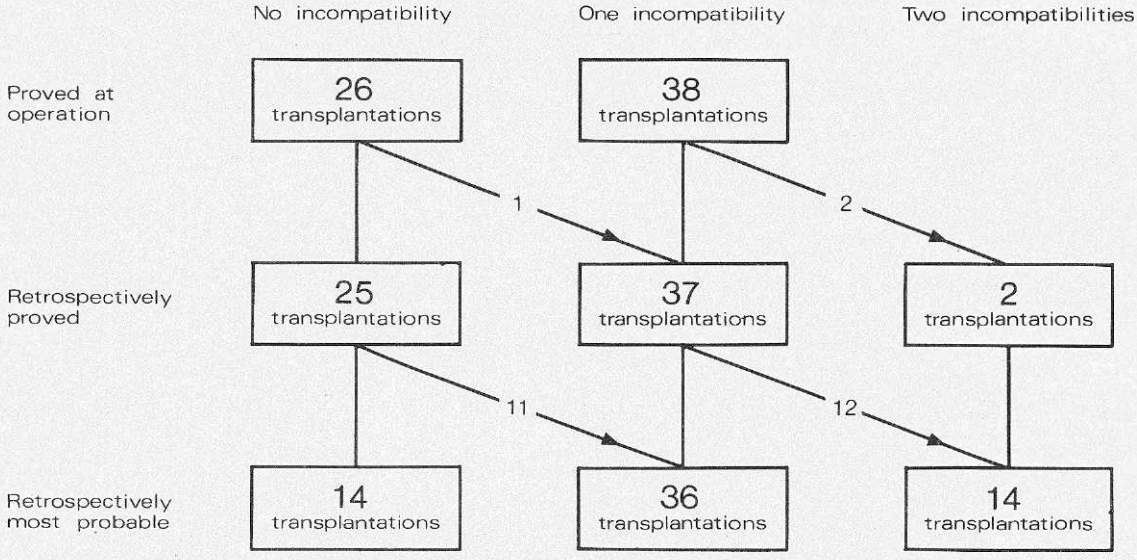

Fig. 9. Degree of HL-A compatibility at the time of operation, retrospectively proved and retrospectively most probable.

The calculations of the most probable compatibility grade includes not only the errors of all statistical calculations but also the following source of error. As some antigens more often seem to be bound together than others it might have been more proper to use the frequencies of haplotypes instead of genes. No such data on the haplotype frequencies were available at the time of this investigation. Subsequent data from other studies (Högman et al., 1972) showed no divergence between haplotype and gene frequencies of importance for the results in this study.

Chapter 5

RESULTS

Overall results

Patient survival

Sixty-three of all 95 patients primarily transplanted with a necro-kidney in Göteborg during the period February 1st 1969 to November 4th 1970 survived for more than one year. Of the 64 patients in this study 43 survived one year (Fig. 10). The patient survival rate was thus 67% in both the groups which indicated that there was no selection of the patients for this particular study from the point of view of patient survival. Among the causes of death (Table VIII) infectious complications were most common. Not only the five cases of septicemia but also the deaths from cerebral hemorrhage, local acute hemorrhage and hemopericardium (the patient was operated on because of an enterococcus endocarditis) were regarded as infectious complications. Fourteen of the patients who died had functioning grafts

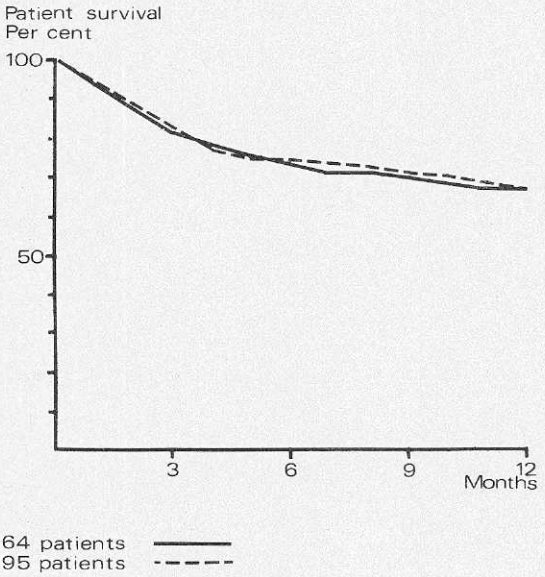

Fig. 10. Patient survival in the series of transplantations investigated and in all 95 transplantations.

Main cause of death	Patient survival time Days
Septicemia	12
Septicemia	17
Septicemia	35
Septicemia	59
Septicemia	189
Cerebral hemorrhage	171
Cerebral hemorrhage	284
Hemopericardium (after open heart surgery)	130
Acute bleeding from grafted site	27
Acute bleeding from grafted site	66
Pancreatitis	54
Pancreatitis	83
Myocardial infarction	28
Heart failure	110
Pulmonary embolism	79
Acute rejection with multiple bleedings	57
Uremia	106
Gastric ulcer with perforation	32
Suicide	252
Carcinoma of the rectum	123
Acute bleeding after retransplantation, four months after removal of first graft	307

Tab. VIII. Main causes of death.

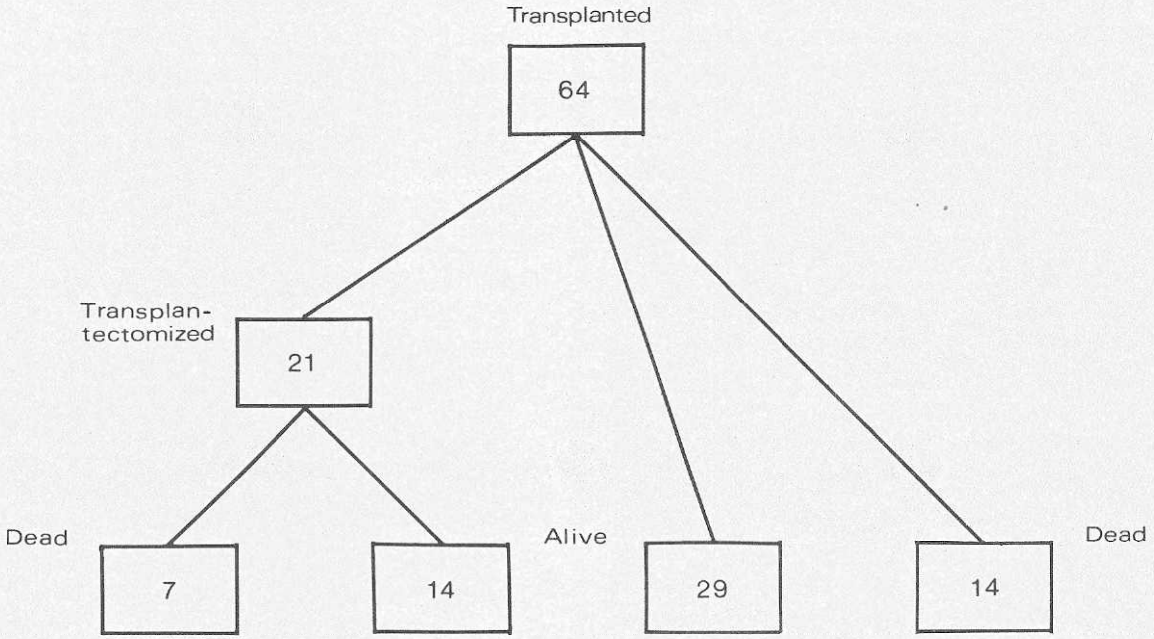

Fig. 11. Overall results.

while the other seven died at various times after transplantectomy (Fig. 11).

Graft survival and function

One year after the transplantation 42 of the 95 primary grafts were still functioning. Twenty-nine of the 64 grafts in this study functioned for more than one year (Fig. 12). These data indicate that there was no selection of the patients or grafts in the investigated material from the point of view of graft survival. Fourteen grafts were lost because of patient death, 21 removed by transplantectomy (Fig. 11). The cause of transplantectomy was irreversible acute rejection in 17 of the 21 cases and acute late bleeding from the grafted site in four cases. All grafts removed because of irreversible rejection showed histological signs of acute cellular rejection. Four of 17 patients undergoing transplantectomy because of irreversible rejection were dead within 12 months. At least three of them died for reasons which cannot be related to the transplantectomy or the rejection (uremia, carcinoma of the rectum and suicide; 92, 119 and 210 days after the transplantectomy) which may indicate that the transplantectomies were not performed too late in the rejection course.

Four of the 21 patients who underwent nephrectomy were retransplanted during the observation period. One of them died because

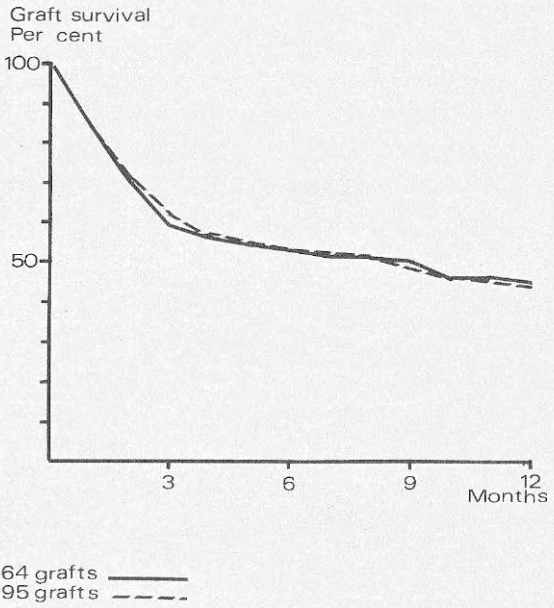

Fig. 12. Graft survival in the series of transplantations investigated and in all 95 transplantations.

of an acute postoperative bleeding seven months after the first transplantectomy. Three patients were retransplanted 2–5 months after the transplantectomy and were still alive at the end of the observation period. In this study only their first grafts were considered and their survival referred to the first graft.

Twenty-one of the 29 patients with grafts surviving for more than 12 months had an unchanged or improved renal function during the observation period. One of these 21 patients who had his own polycystic kidneys left had proteinuria, all the others had a protein excretion of less than 700 mg/day.

Three patients had a slightly decreasing renal function during the observation time and proteinuria which possibly were signs of chronic rejection. Five patients had definite chronic rejections with decreasing renal function and proteinuria. No patient with a remaining graft required dialysis treatment.

The grafts from the eight patients with decreasing renal function and proteinuria and from the 17 patients subjected to transplantectomy due to irreversible rejection were thoroughly investigated for signs of recurrent glomerulonephritis. Of these 25 patients eight had glomerulonephritis as the primary disease while 12 had chronic pyelonephritis. This was the same ratio as in the total material of this study. The clinical course and the histopathological findings excluded recurrent glomerulonephritis in all but one case in which the diagnosis may be regarded as possible. In this study this transplantectomy was accepted as due to irreversible rejection.

	First 6 months postoperatively Per cent	7th-12th month postoperatively Per cent
No rejection	55	83
One or more rejections, serum creatinine again ≤ 2.0 mg%	21	0
One or more rejections, serum creatinine ≥ 2.1 mg% afterwards	24	17

Tab. IX. Frequencies of reversible rejection episodes (29 transplantations with graft surviving one year).

Of the 29 grafts functioning for more than 12 months 16 (55%) had no clinical rejection episodes at all during the first 6 postoperative months (Table IX). The other 13 had one or more acute rejection episodes, six with good restitution of the kidney function, seven with remaining but impaired function. During the second postoperative half-year 24 had no signs of rejection and five had rejection episodes with definite reduction of the kidney function.

Graft survival related to donor factors, recipient factors and HL-A compatibility

Donor factors

Many conditions in the donor are of importance for the viability of the kidney and may also be of importance for the immunogenicity of the graft. The degree of viability per se may have an immunological significance.

It is not known which factors in the premortem phase of the donor are most important for organ viability, but factors such as blood pressure, organ perfusion, body temperature and the nature of trauma or disease are thought to be of great importance. Reliable data on relevant parameters in the premortem phase of the donors are however often impossible to obtain retrospectively. This investigation was therefore concentrated on an evaluation of the influence of age and cause of death of the donors and of the duration of warm and cold ischemia times on the outcome of the transplantation.

The graft survival rate was therefore correlated to the age of the donor. Three age groups were considered; up to 30 years, 30–50 years and above 50 years of age (Fig. 13). The graft survival rate was found to be higher if the donor was older than 50 years. The influence of the diagnosis of the donor was also analysed. The graft survival rate was not found to be correlated to the disease or trauma of the donor.

The graft survival rate was correlated to four different lengths of warm ischemia time, 0–20

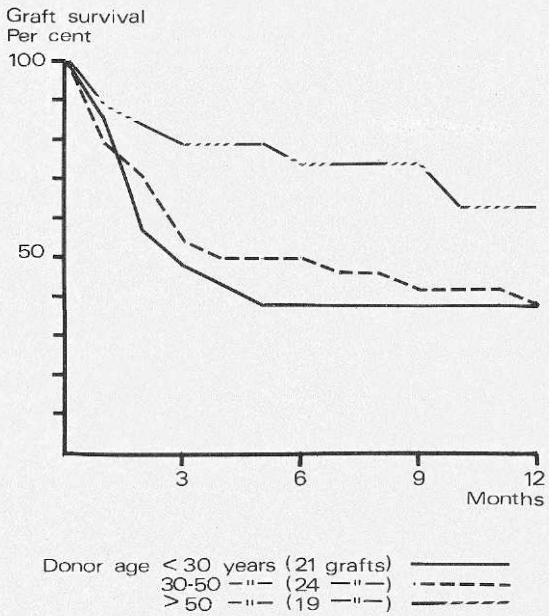

Fig. 13. Graft survival correlated to age of the donor.

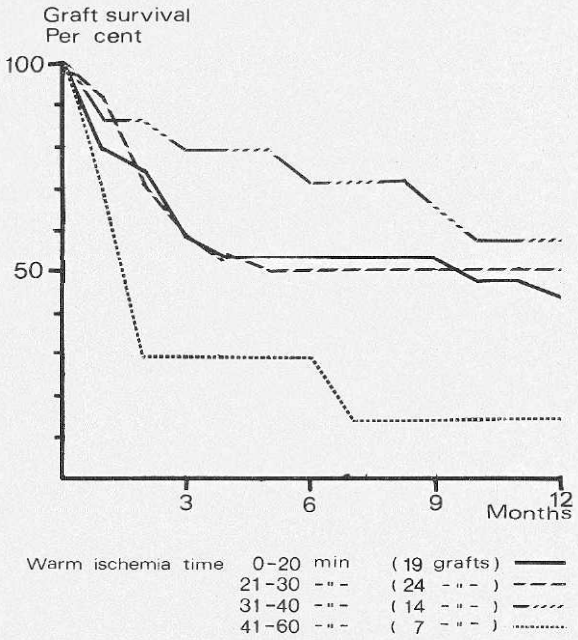

Fig. 14. Graft survival correlated to duration of the warm ischemia.

minutes, 21–30, 31–40 and 41–60 minutes (Fig. 14). The graft survival was poor if the warm ischemia time exceeded 40 minutes. The differences in graft survival rate were small if the warm ischemia time was below 40 minutes; a somewhat higher graft survival rate was found with a warm ischemia time of 31–40 minutes.

An influence of the cold ischemia on the graft survival rate was found when the survival rate was correlated to different duration of cold ischemia, less than 6 hours, 6–10 hours and more than 10 hours (Fig. 15). The shorter the cold ischemia time the better was graft survival rate at one year. With a cold ischemia time of more than 10 hours the one year survival rate was only 35% with the preservation method used in this series.

The ischemic damage of the graft, occurring during the pre- and postmortem phase of the donor, often gave rise to an oliguric or anuric period in the transplant. The duration of such a period may to some extent reflect the severity of the ischemic damage; however also other factors, including immunological ones, may influence the duration of such an oligo-anuric period. In this series of transplantations an early start of function did not result in any significant increase of the one year graft survival rate compared to a late start of

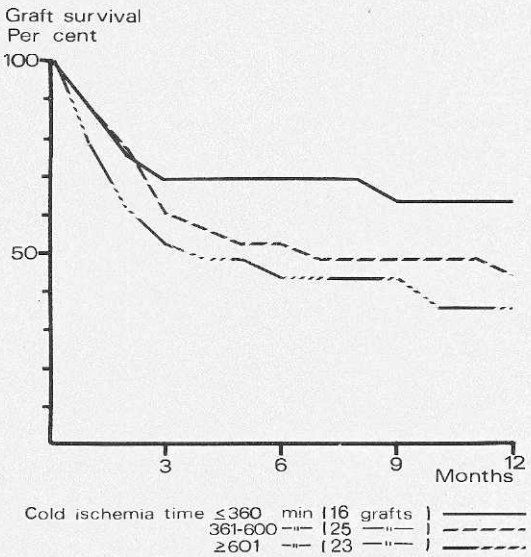

Fig. 15. Graft survival correlated to duration of the cold ischemia.

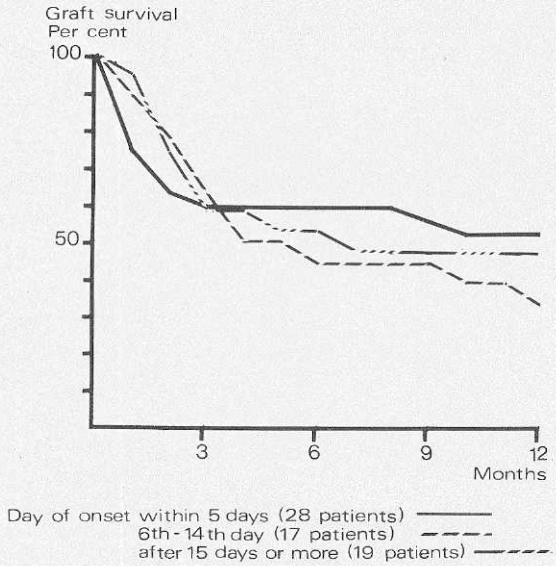

Fig. 16. Graft survival correlated to day of onset of renal function.

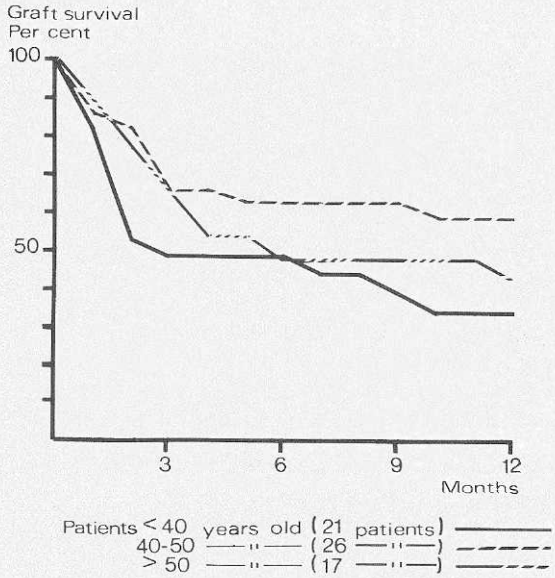

Fig. 17. Graft survival correlated to age of the recipient.

function of the graft (Fig. 16).

This analysis emphasizes the influence of the duration of warm and cold ischemia and of the age of the donor for the graft survival.

Recipient factors

Among factors in the recipient possibly affecting the results of kidney transplantation special interest was focused on the recipient's age, cause and stage of uremia and the given immunosuppressive therapy. The graft survival rate was correlated to three different recipient age groups: up to 40 years, 40–50 years and above 50 years of age. The age of the recipient was found to influence the graft survival (Fig. 17). The highest graft survival rate one year after transplantation was found among recipients in the age of 40–50 years, the lowest among recipients below the age of 40 years.

The original disease of the recipients was found to be of significant importance for the graft survival (Fig. 18). One year after the transplantation the graft survival rate for recipients with glomerulonephritis was twice as high as the rate for recipients with pyelonephritis. The graft survival rate for recipients with polycystic kidney disease was somewhat lower than for recipients with glomerulonephritis.

The influence of the duration of preoperative dialysis treatment on graft survival was investigated. The graft survival rate was calculated for recipients transplanted before initiation of dialysis treatment, for recipients dialysed for less than three months, 3–12 months and for recipients dialysed for more than one year. A moderate influence of the duration of preoperative dialysis on the graft

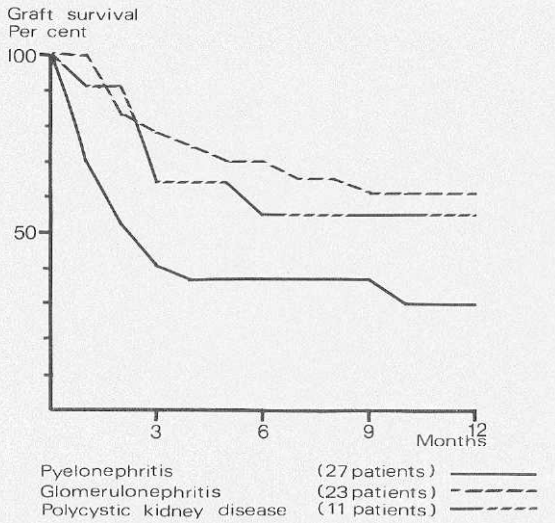

Fig. 18. Graft survival correlated to cause of uremia of the recipient.

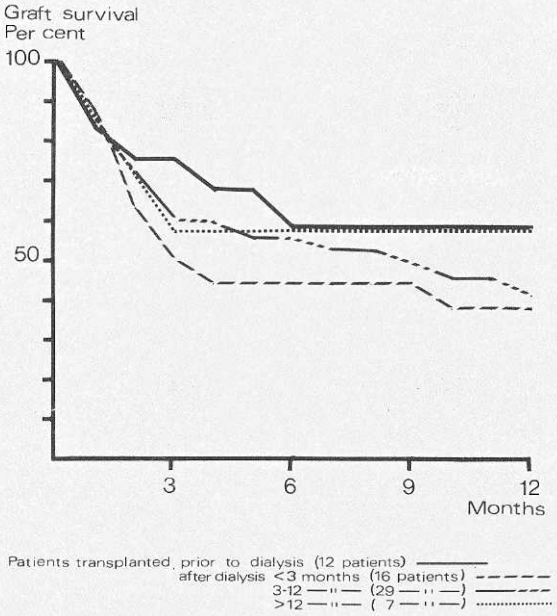

Fig. 19. Graft survival correlated to duration of the preoperative dialysis.

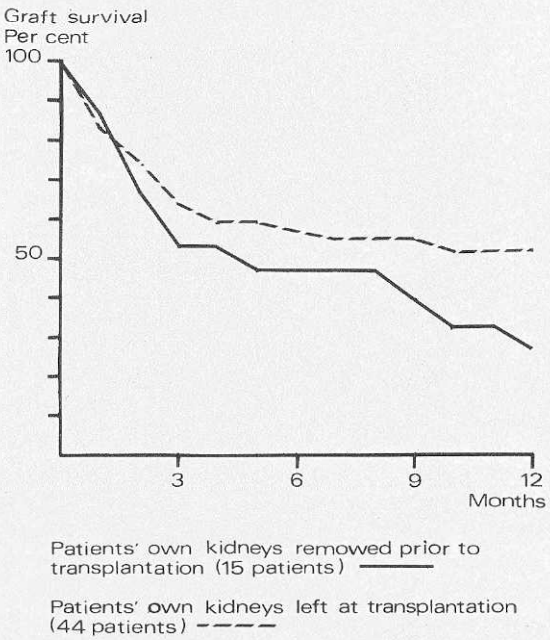

Fig. 20. Graft survival in transplantations of recipients with own kidneys left or removed (last own kidney).

survival was found (Fig. 19). The highest graft survival rate was found among recipients transplanted in a predialytic phase or after dialysis treatment for more than one year. The duration of the preoperative dialysis may also influence the frequency of preformed HL-A antibodies in the recipient provoked by blood transfusions. In this series of patients nine had developed HL-A antibodies before the transplantation. Two of these nine grafts survived one year.

The influence of recipient nephrectomy was also analysed (Fig. 20). A better graft survival was found one year after the transplantation in the larger group of recipients with their own kidneys left at the time of transplantation than in the group nephrectomized before the transplantation.

The immunosuppressive therapy was well standardized and all recipients were given the same prednisolone dose (see page 16). Depending on the bone marrow depressing effect of azathioprine some of the patients however received a lower azathioprine dose than that intended. This was the case of 23 patients who tolerated a mean daily dose of 1.7

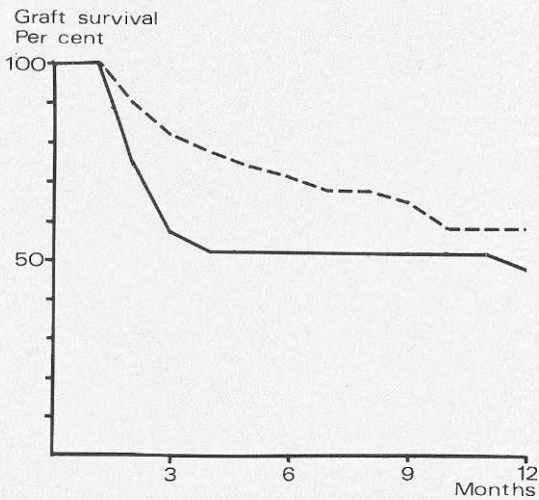

Fig. 21. Graft survival correlated to the size of the azathioprine dose given during the first postoperative month.

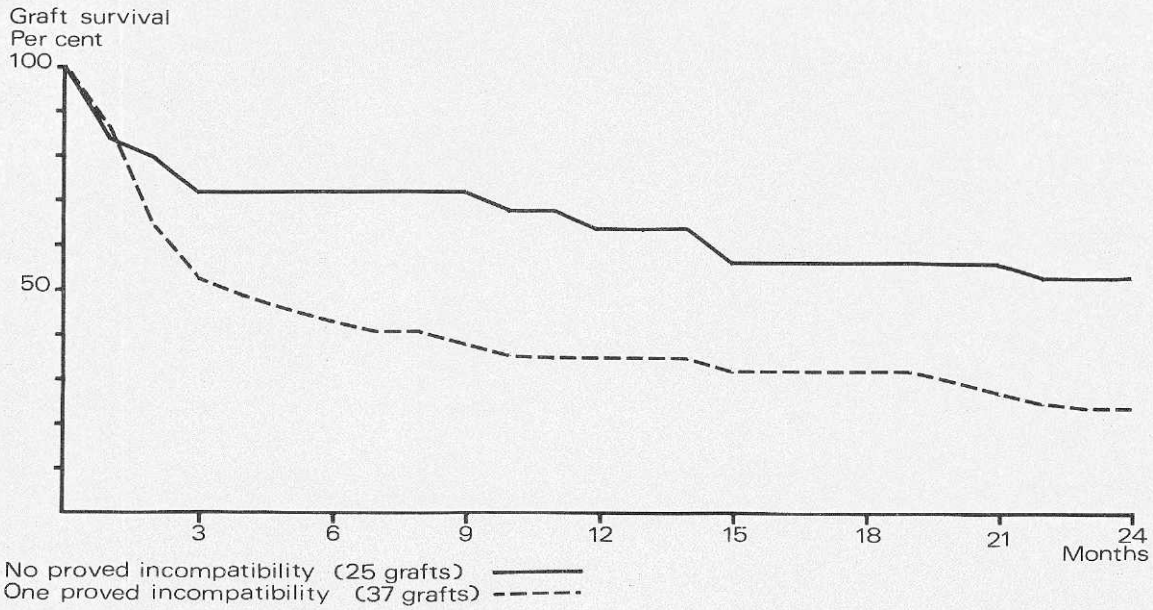

Fig. 22. Graft survival correlated to retrospectively proved compatibility grade.

mg/kg bodyweight during the first postoperative month, while 31 patients received the intended dose, i.e. a mean daily dose of 2.9 mg/kg. The influence of different immunosuppressive therapy on graft survival was analysed (Fig. 21). Of the 64 transplantations in this series 54 grafts survived for more than one month. The graft survival rate of the 31 recipients tolerating the higher azathioprine dose was higher than the graft survival rate for recipients who did not tolerate the intended azathioprine dose. This difference was most evident during the first months but was still present one year after the transplantation.

This analysis emphasizes the influence of the recipient's age and cause of uremia, the duration of preoperative dialysis treatment as well as the influence of recipient nephrectomy and the azathioprine dose given on graft survival.

Histocompatibility

The influence of compatibility and incompatibility in the HL-A system was analysed according to the different degree of compatibility defined in chapter IV, i.e. considering 1) proved degree of HL-A incompatibility and 2) most probable degree of HL-A incompatibility. The survival rate of grafts with no proved incompatibility was found to be significantly higher than the survival rate of grafts with one proved incompatibility (Fig. 22). The differences in graft survival rates increased during the first six months and then remained unchanged up to two years after the transplantation.

The survival rate of grafts with no probable incompatibility was even higher (Fig. 23). There was however no difference in graft survival rate if there existed one or two probable incompatibilities.

These data emphasize that compatibility in the HL-A system was important for the graft survival.

As other factors both in the donor and in the recipient also were found to be of importance for graft survival these factors might be responsible for the different survival rates of compatible and incompatible grafts. The distribution of such factors of the donor and of the recipient which favoured graft survival, "positive factors", were therefore calculated within the groups of compatible-graft-transplantations and compared to the distribution of these "positive factors" in the total material (Table X). No differences were found

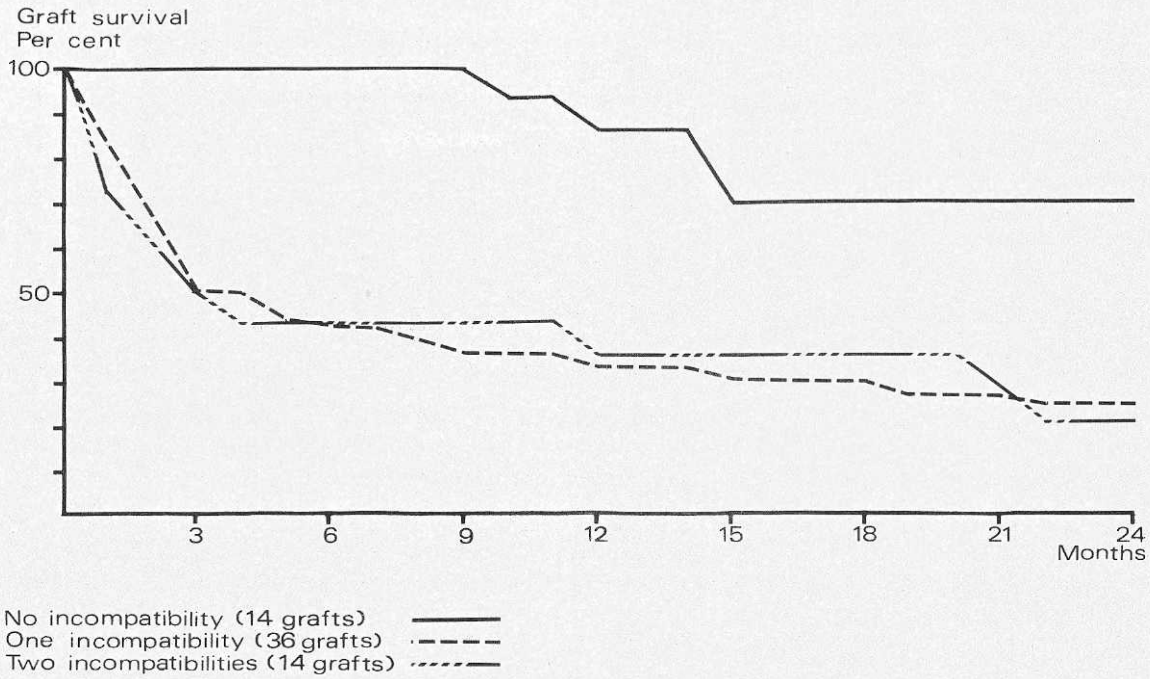

Fig. 23. Graft survival correlated to retrospectively most probable compatibility grade.

	Per cent of total material (64 patients)	Per cent of patients with no proved incompatibility (25 patients)	Per cent of patients with most probably no incompatibility (14 patients)
Age of recipient 40-50 years	41	52	50
Glomerulonephritis as cause of uremia	36	36	36
Transplanted predialytic	19	32	50
Transplanted after dialysis >12 months	11	8	7
Own kidneys left at transplantation	69	76	86
Mean daily azathioprine dose 2.9 mg/kg (to patients with grafts surviving one month)	57	44	60
Age of donor >50 years	30	32	29
Warm ischemia ≤ 40 minutes	89	92	100
Warm ischemia 31-40 minutes	22	16	14
Cold ischemia ≤ 600 minutes	64	64	64
Cold ischemia ≤ 360 minutes	25	32	29

Tab. X. Frequencies of "positive factors" in all transplantations and in transplantations of grafts with no proved or no probable HL-A incompatibility.

in the distribution of "positive factors" with one exception. There was a higher frequency of patients transplanted in a predialytic phase among those with "no incompatibility". This was expected since patients accepted for transplantation in a predialytic phase were more often transplanted only if a kidney with good compatibility could be offered. This moderate difference could however have had only minor importance for the better survival rates of compatible grafts. As otherwise no significant differences existed in frequencies of "positive factors" it was evident that no selection was made of the grafts or of the patients for transplantation with HL-A compatible kidneys.

HL-A compatibility was therefore accepted as a favourable factor. Which of the "positive factors" were then the most important one? To investigate this the frequencies of all "positive factors" found were compared in two groups of transplantations, one with grafts surviving for more than 12 months and another with grafts surviving for less than 12 months (Table XI). As expected higher frequencies of "positive factors" were found in the group of transplantations with grafts surviving for more than 12 months. Also the frequencies of all other investigated factors, not previously found to be "positive", were calculated, but none of these factors was found in a higher frequency among grafts surviving more than 12 months than among grafts surviving less than 12 months.

The difference in frequencies of each "positive factor" could be taken as a measure of the relative importance of just that factor. The statistical calculation of these differences gave significant differences only concerning the compatibility factors ($p<0.01$ if no incompatibility was proved, $p<0.001$ if no incompatibility was probable). The differences of frequencies of the other "positive factors" were not statistically significant.

This implies that the most important single factor for graft survival was compatibility in the HL-A system between donor and recipient.

	Per cent of 29 patients with graft surviving > 12 months	Per cent of 35 patients with graft surviving < 12 months
Age of donor >50 years	41	20
Warm ischemia ≤40 minutes	97	83
Cold ischemia ≤600 minutes	72	57
Cold ischemia ≤ 360 minutes	35	17
Age of recipient 40-50 years	52	32
Glomerulonephritis as primary disease	48	26
Transplanted predialytic	21	17
Transplanted after dialysis >12 months	14	9
Own kidneys left at transplantation	80	60
Daily azathioprine dose 2.9 mg/kg	62	52
No proved incompatibility	55	26
No probable incompatibility	41	6

Tab. XI. Frequencies of "positive factors" in transplantations of grafts surviving and not surviving one year.

	First 6 months postoperatively		7th-12th months postoperatively	
	No rejection episode Per cent	Reversible rejection(s) Per cent	No rejection episode Per cent	Reversible rejection(s) Per cent
No proved incompatibility (16 patients)	56	44	88	12
One proved incompatibility (13 patients)	38	62	77	23

Tab. XII. Frequencies of reversible rejection episodes correlated to proved compatibility grade (29 transplantations of grafts surviving one year).

	First 6 months postoperatively		7th-12th months postoperatively	
	No rejection episode Per cent	Reversible rejection(s) Per cent	No rejection episode Per cent	Reversible rejection(s) Per cent
Most probably no incompatibility (12 patients)	67	33	83	17
Most probably one or two incompatibilities (17 patients)	35	65	82	18

Tab. XIII. Frequencies of reversible rejection episodes correlated to most probable compatibility grade.

The great importance for graft survival of HL-A compatibility should be expected to be reflected in a lower frequency and/or severity of rejection episodes. An analysis was therefore made of the frequencies of reversible and irreversible rejections in HL-A compatible and HL-A incompatible grafts. The frequency of reversible rejection episodes was slightly lower in the compatible grafts than in the incompatible grafts but the groups were small (Table XII, Table XIII).

The frequency of irreversible rejections was however the same in grafts with no proved incompatibility as in grafts with one proved incompatibility (Table XIV). Only the smaller group of grafts with most probably no incompatibility had a lower frequency of irreversible rejections (Table XV) compared to the groups of grafts with most probably one or two incompatibilities.

	Irreversible rejection No	Per cent
No proved incompatibility (25 patients)	7	28
One proved incompatibility (37 patients)	9	24

Tab. XIV. Transplantectomies due to irreversible rejections correlated to proved compatibility grade.

	Irreversible rejection No	Per cent
Most probably no incompatibility (14 patients)	2	14
Most probably one incompatibility (36 patients)	11	31
Most probably two incompatibilities (14 patients)	4	29

Tab. XV. Transplantectomies due to irreversible rejections correlated to most probable compatibility grade.

Thus another reason for graft loss must be responsible for the different survival rates of compatible and incompatible grafts. Other reasons for graft loss were arterial bleeding (four grafts) and loss of patient with remaining graft (14 grafts). The distribution of patients who died with functioning grafts was therefore further analysed. Only one of the 25 recipients transplanted with HL-A compatible grafts died with a functioning graft as compared to 13 of 37 recipients (35%) transplanted with a graft with one proved incompatibility (Table XVI). No patient transplanted with a most probably HL-A compatible graft died with a functioning graft while 31% respectively 21% of patients transplanted with grafts with probably one or two incompatibilities died with remaining functioning grafts (Table XVII).

Summary: *Many factors in the donor and in the recipient were important for graft survival. The most important single factor for graft survival was HL-A compatibility. Incompatibility led to a higher rate of graft loss mainly because of a higher recipient mortality rate.*

These findings required a further analysis of factors influencing the patient survival.

	Dead with functioning graft	
	No	Per cent
Retrospectively no proved incompatibility (25 patients)	1	4
Retrospectively one proved incompatibility (37 patients)	13	35

Tab. XVI. Mortality of recipients of grafts with no or one proved incompatibility correlated to graft function at death (excluded is one patient dead after retransplantation seven months after primary transplantation).

Patient survival related to donor factors, recipient factors and HL-A compatibility

The importance for patient survival of the same factors previously investigated for graft survival influence was analysed.

Donor factors

The age of the donor was not found to influence patient survival at one year (Fig. 24). The influence of the cause of death of the donor was analysed but different survival rates were not found in the different diagnostic groups.

There was only a small difference in patient survival on the basis of the duration of the warm ischemia time if this did not exceed 40 minutes (Fig. 25). The best survival rate was obtained among recipients receiving a graft with a warm ischemia time of 31–40 minutes.

The patient survival rate was found to be higher when the cold ischemia time did not exceed 6 hours (Fig. 26).

These data emphasize the influence of the duration of warm and cold ischemia on patient survival.

Recipient factors

The age of the recipient was found to be important for patient survival. Recipients in the ages of 40–50 years had the highest survival rate and recipients in the ages below 40 years the lowest (Fig. 27).

	Dead with functioning graft	
	No	Per cent
Retrospectively most probably no incompatibility (14 patients)	0	0
Retrospectively most probably one incompatibility (35 patients)	11	32
Retrospectively most probably two incompatibilities (14 patients)	3	21

Tab. XVII. Mortality of recipients of grafts with and without probable incompatibility correlated to graft function at death (excluded is one patient dead after retransplantation seven months after primary transplantation).

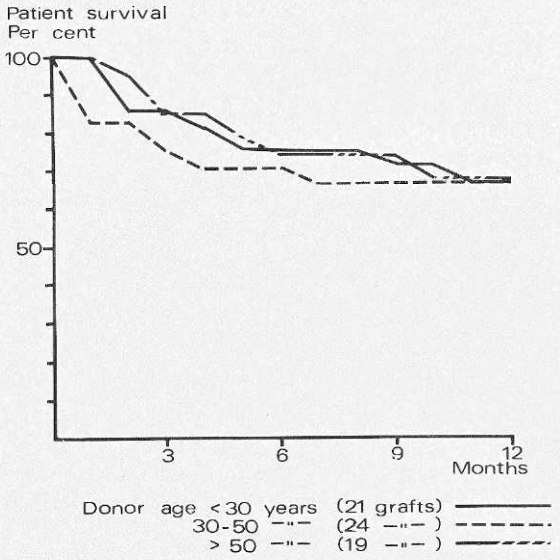

Fig. 24. Patient survival correlated to age of the donor.

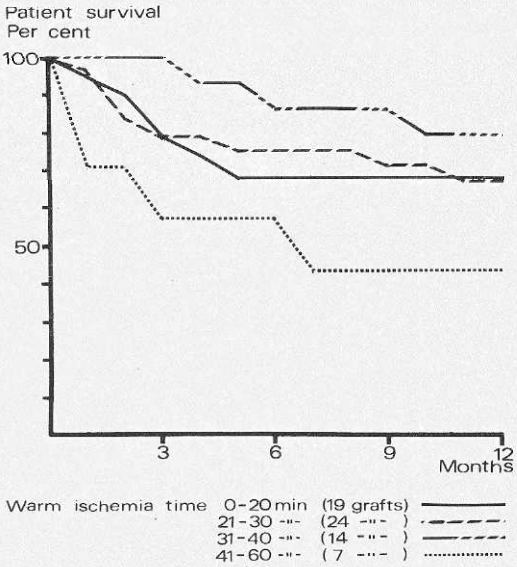

Fig. 25. Patient survival correlated to duration of the warm ischemia.

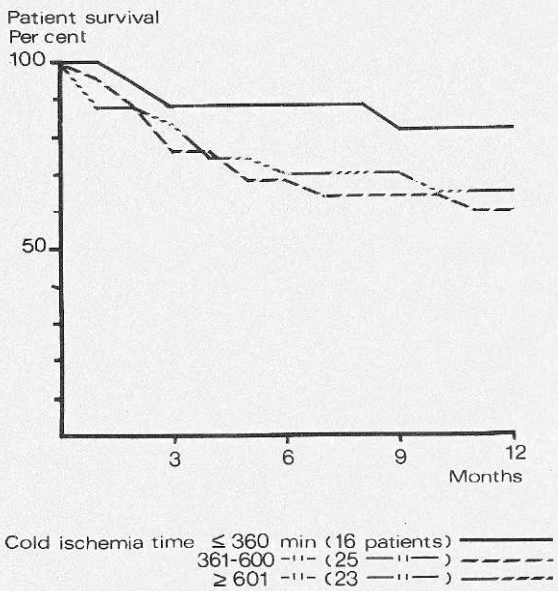

Fig. 26. Patient survival correlated to duration of the cold ischemia.

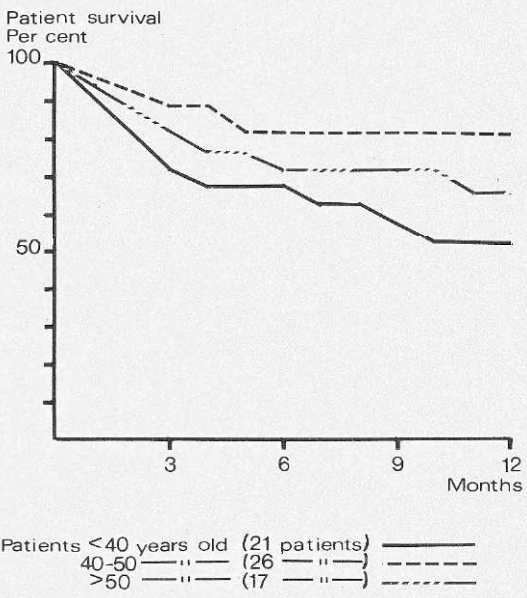

Fig. 27. Patient survival correlated to age of the recipient.

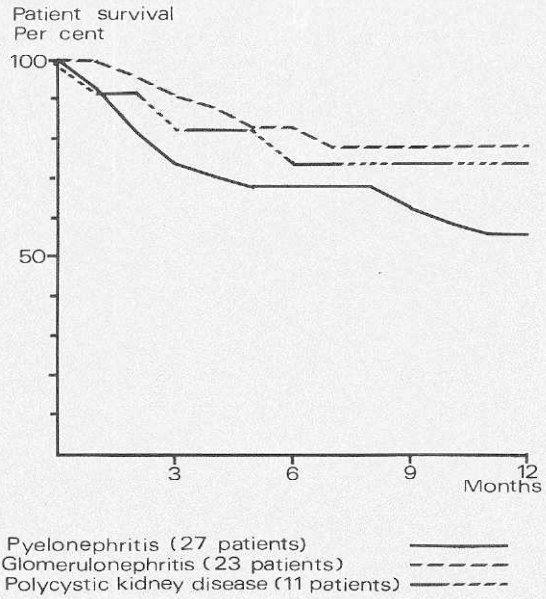

Fig. 28. Patient survival correlated to cause of uremia of the recipient.

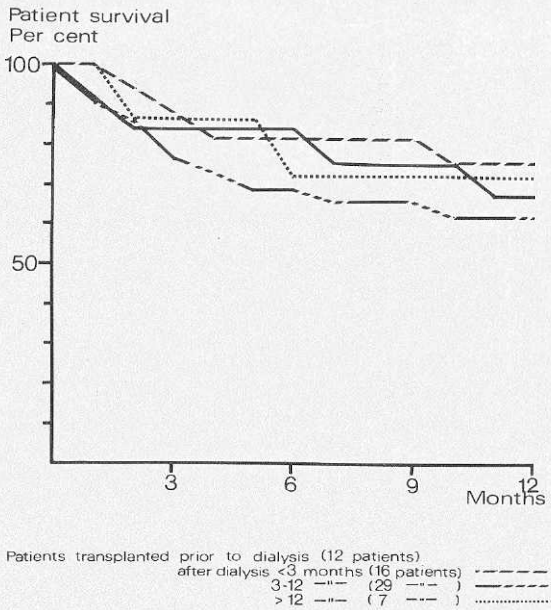

Fig. 29. Patient survival correlated to duration of the preoperative dialysis.

The original disease of the recipient was found to be important. Recipients with pyelonephritis had a lower survival rate than recipients with glomerulonephritis or polycystic kidney disease (Fig. 28). The duration of preoperative dialysis treatment was not found to have any significant importance for patient survival (Fig. 29).

There was no difference in survival rates observed if the recipients were nephrectomized before transplantation or not (Fig. 30).

As described earlier, the immunosuppressive therapy was well standardized. Some recipients however did not tolerate the intended azathioprine dose so that they therefore received a lower dose. The lower azathioprine dose was given to 23 of the 54 recipients with grafts functioning for more than one month; the mean daily dose during the first postoperative month was 1.7 mg/kg bodyweight. The other 31 received a mean daily dose of 2.9 mg/kg. The patient survival rate was found to be higher for recipients receiving the lower dose (Fig. 31).

This analysis emphasizes the influence of the recipient's age, cause of uremia and of the given azathioprine dose on patient survival.

Histocompatibility

The patient survival rate was found to be 88% at one year when the patient was transplanted with a kidney with no proved HL-A incompatibility compared to only 51% if one incompatibility was proved (Fig. 32).

When the most probable compatibility grade was considered no mortality was found if the patients were transplanted with HL-A compatible grafts (Fig. 33). The mortality rate was 39% if one incompatibility was probable and 57% if two incompatibilities were probable.

This significant difference in patient survival rate might be explained by other "positive factors" if they were present more frequently in the groups with no incompatibility. The frequencies of these "positive factors" were therefore analysed in the two groups of transplantations with proved or probable HL-A compatible grafts and compared to the frequencies of "positive factors" in the total

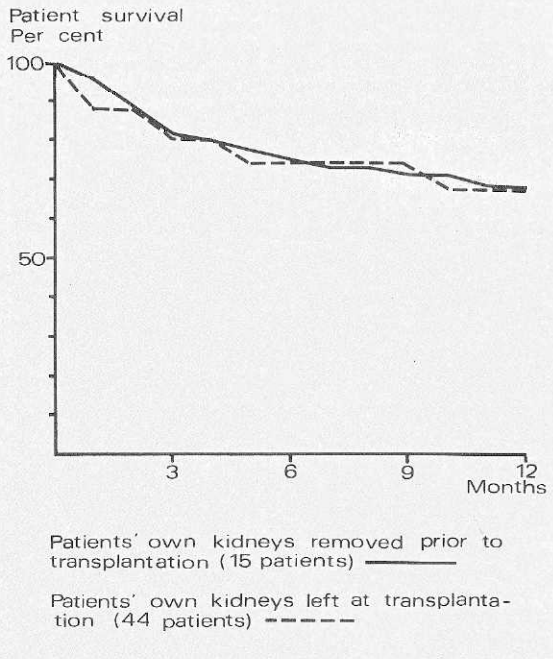

Fig. 30. Patient survival in transplantations of recipients with own kidneys left or removed (last own kidney).

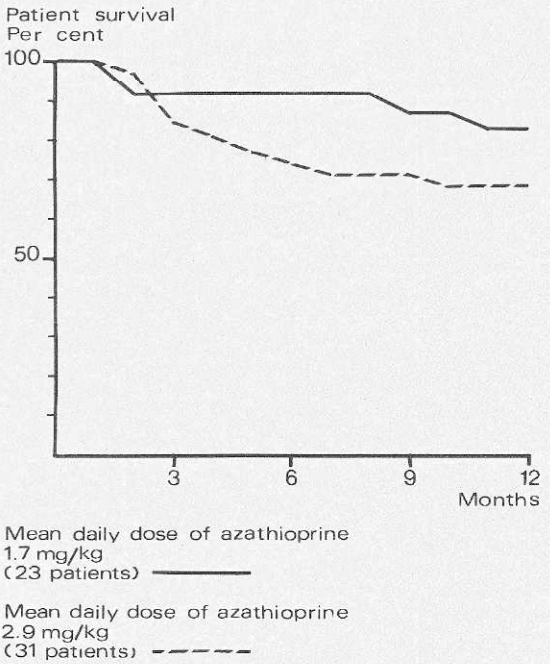

Fig. 31. Patient survival correlated to the size of the azathioprine dose given during the first postoperative month.

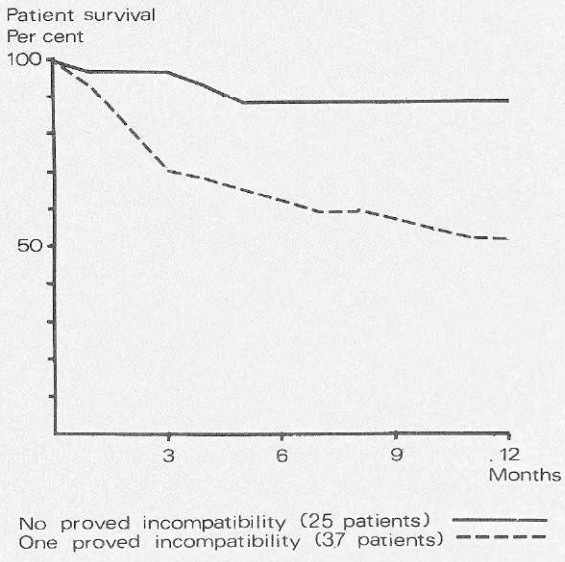

Fig. 32. Patient survival correlated to retrospectively proved compatibility grade.

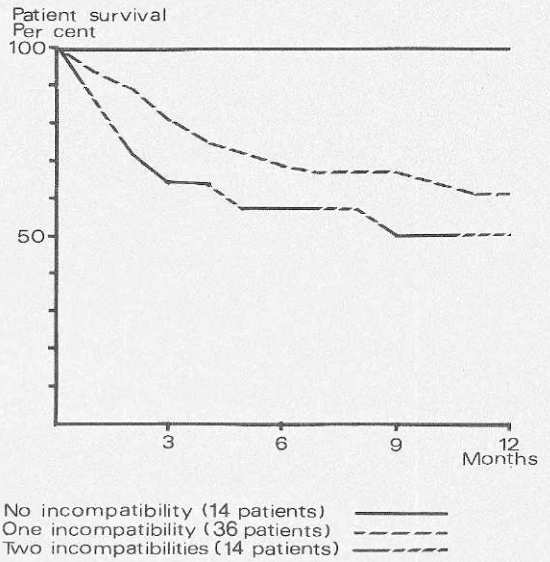

Fig. 33. Patient survival correlated to retrospectively most probable compatibility grade.

	Per cent of all 64 transplantations	Per cent of 25 transplantations with no proved incompatibility	Per cent of 14 transplantations with most probably no incompatibility
Warm ischemia 31-40 minutes	22	16	14
Cold ischemia ≤ 360 minutes	25	32	29
Age of recipient 40-50 years	41	52	50
Glomerulonephritis as primary disease	36	36	36
Mean daily azathioprine dose 1.7 mg/kg (to patients with grafts surviving one month)	43	52	43

Tab. XVIII. Frequencies of "positive factors" in all transplantations and in transplantations of grafts without proved or probable incompatibility.

material (Table XVIII). No differences were found which implied that compatibility in the HL-A system was of significant importance for patient survival.

To investigate which factors were most important for patient survival the frequencies of "positive factors" were calculated in the group of 43 patients who survived for more than 12 months and in the group of 20 patients who died within one year (Table XIX). The differences in frequencies were measures of the importance of each factor. The statistical calculation of these differences showed significant differences in proved compatibility rate and in probable compatibility rate ($p<0.005$) while the other differences were not statistically significant.

Summary: *Among the factors of importance for patient survival after necrokidney transplantation compatibility in the HL-A system between donor and recipient was found to be the most important single factor.*

	Per cent of 43 patients surviving one year	Per cent of 20 dead patients
Warm ischemia 31-40 minutes	23	15
Cold ischemia ≤ 360 minutes	30	15
Age of recipient 40-50 years	49	20
Glomerulonephritis as primary disease	42	25
Mean daily azathioprine dose 1.7 mg/kg	44	30
No proved incompatibility	51	10
Most probably no incompatibility	33	0

Tab. XIX. Frequencies of "positive factors" in transplantations of patients surviving one year and in transplantations of patients who died.

Chapter 6

CONSIDERATIONS OF THE IMPORTANCE OF DIFFERENT FACTORS FOR GRAFT AND PATIENT SURVIVAL

When evaluating a clinical method there is always a multiplicity of factors which influence the end results and have to be considered. This is especially true for the method of necrokidney transplantation. The great number of important factors in the donor and in the recipient and the complex immunological reactions and their suppression makes it especially difficult to evaluate the importance of *one* specific factor. The effect of variations of *one* factor will easily be hidden by the effects of simultaneous variations of other factors. Although the main purpose of this study was to evaluate the influence of compatibility in the HL-A system between donor and recipient, it was necessary first to find and evaluate other factors of importance for the clinical outcome.

The clinical method of transplantation, regarding patient and graft selection, immunosuppressive regimen etc. varies at different transplantation centres which makes a comparison of results between different centres impossible. It therefore became necessary first to evaluate the importance of some relevant factors in the donors and in the recipients of this specific transplantation series. The parameters studied were chosen on the basis of known and expected importance for graft and patient survival. The factors studied were some of the most important according to our own experience and that of others, but certainly not all which might influence the results of transplantation.

The material in this study was chosen to provide a uniformely treated patient series. Also other factors e.g. graft preservation technique were standardized as far as possible. Therefore this series of transplantations was considered very suitable for a study of the effect of various factors on the result of transplantation. Several factors were found to be of importance for graft and patient survival. One way to diminish the multiplicity of relevant factors when studying the effect of one or few factors was to divide the recipients into smaller, less heterogeneous groups. The disadvantages of such a method were obvious. Too small groups of patients would not allow for well founded conclusions. Instead of performing such selections another method was chosen. The influences of various factors on the end results, i.e. on the graft and patient survival rates were studied on the whole series of patients. Different quantities or qualities of every factor studied were correlated to the graft survival rate and to the patient survival rate. Independent of the actual validity of just one defined factor the group of patients with that factor had a certain survival rate. Some factors were found to be ''positive factors'' for survival. A comparison was then made of the frequencies of these ''positive factors'' in two different groups of patients, one with graft survival (or patient survival) more than 12 months and another with graft survival (or patient survival) less than 12 months. It was consistent to find a higher frequency of ''positive factors'' in the group of patients with

(graft) survival than in the group of patients not surviving or with grafts not surviving. The differences in frequencies of these factors were measurements of the importance of the individual factor. In the following the different graft factors which were found to influence the graft and patient survival will be considered first, then the different recipient factors and finally the HL-A-compatibility grade.

Graft factors

The biological event within a graft before the transplantation both in the donor and during the extracorporeal handling has been thought to have importance mainly for the viability of the organ, but may also have importance for the immunogenicity of the graft.

Viability of the graft

Donor factors

The earlier history of the donor was often unknown or very badly known. The risks of lower viability of the kidney due to earlier diseases must be considered small though possible. A more important factor might have been the premortem phase of the donor. During a protracted premortem phase an insufficient perfusion of the kidney exists for shorter or longer time. The most ideal donor situation when nephrectomy can be performed on a donor with maintained artificial respiration and circulation was very rare in this material. Some donors suffered a sudden death; others had a period of maximal premortal stress with release of vasoactive substances. Others were heavily treated during resuscitation attempts with vasoactive and cardiotropic drugs for a shorter time before the donor nephrectomy. Others were ''treated'' for days with artificial respiration though braindead, awaiting spontaneous cardiac arrest, which then often occurred at a body temperature of about 28°C after an oliguric-anuric period of several days.

The premortem factors were too complex and too badly registered to allow a proper evaluation in this series.

Organs from young donors have been regarded as more resistent to ischemic damage than organs from old donors. It was therefore surprising to find a higher graft survival rate when the donor age was above 50 years (Fig. 13). The reason for this finding might be a more critical selection of older donors, a better capacity to resist premortally released vasoactive or toxic substances, a decreased immunogenicity or an unequal distribution of other favourable factors. Whatever may be the reason the group of recipients with grafts from donors over the age of 50 years had a higher graft survival rate than the group of recipients with grafts from younger donors. The patient survival rate was however not influenced by the age of the donor (Fig. 24).

Preservation

The duration of ischemia periods and the perfusion technique are known to have great influence on the kidney viability. The perfusion technique used in this material was well standardized and with two exceptions the same perfusates were used in all grafts. The carefulness of the initial perfusion may have some importance but only small variations in this aspect were expected.

The viability ought to be better the shorter the warm ischemia time. Despite this the graft survival rate was not superior if the warm ischemia time was less than 20 minutes compared to an ischemia time of 21–40 minutes (Fig. 14). Except for an unequal distribution of recipients in the groups, this could be explained by the fact that a careful proper nephrectomy technique prolongs the warm ischemia time but protects the kidney from traumatic damage. One important observation is that very short ischemia times were very rare in this material. Warm ischemia time exceeding 40 minutes resulted in a low graft survival rate.

A direct relation between the duration of the cold ischemia and the rate of graft loss was noted (Fig. 15).

The duration of the warm and cold ischemia periods influenced not only the graft survival

but also the patient survival. This might be an effect of a prolonged decreased graft function or an effect of changed immunological conditions due to the ischemic damage. One consequence of long ischemia times was observed which might have influenced the graft and patient survival. There was a higher frequency of postoperative local infectious complications (wound infections and urinary infections) in the recipients receiving grafts with prolonged ischemia times (Fig. 34, Fig. 35).

The length of the warm ischemia and cold ischemia times had importance for the duration of the postoperative oliguric-anuric period. The duration of this period could therefore be taken as a measure of the degree of ischemic damage. In addition other factors were important for the duration of this period, e.g. rejection episodes and postoperative local complications. The small variations in graft survival according to "day of onset" were therefore of minor interest.

Immunogenicity of the graft

Very little is known about factors influencing the immunogenicity of the graft. Earlier disease, the age of the donor as well as damage to the kidney, pre- or postmortally might be important. The perfusion technique may at least have importance in that an improper perfusion or an improper perfusate leaves passenger leukocytes of immunological significance (Stuart et al., 1971 a and b). The immunogenicity of different HL-A antigens and HL-A compatibility grade will be discussed below.

Recipient factors

Various conditions in the recipient had importance either of immunological signifi-

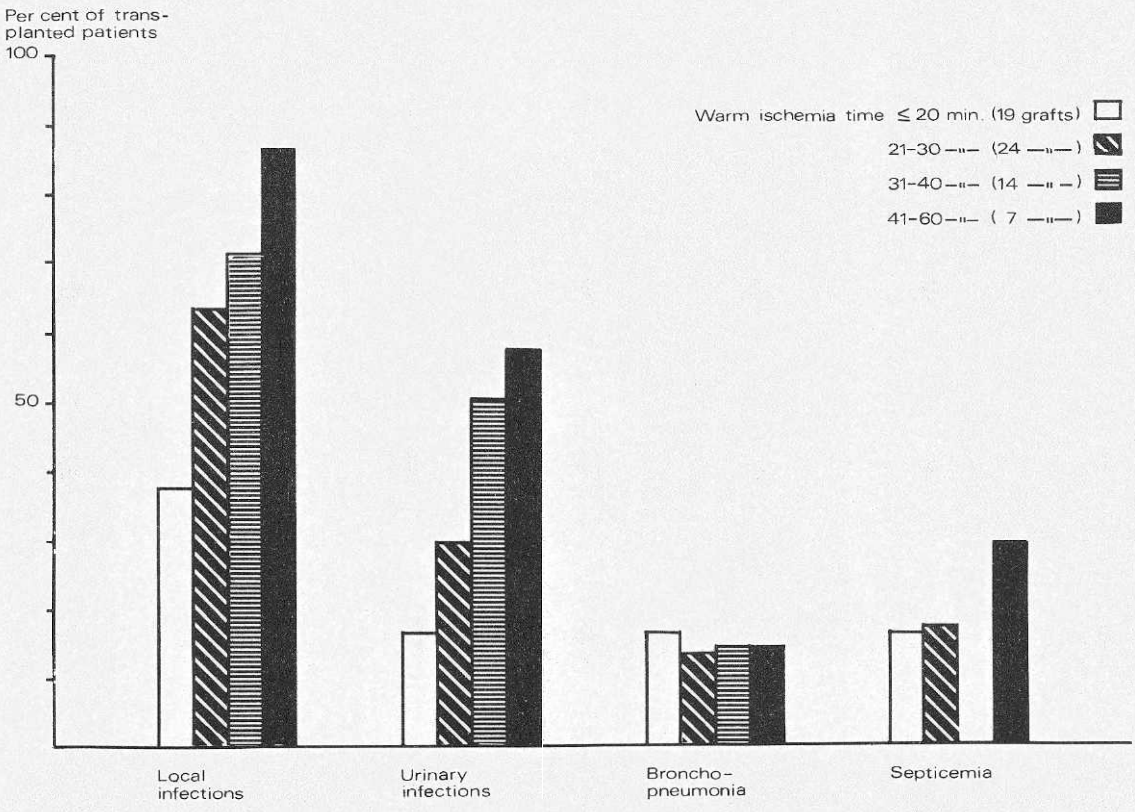

Fig. 34. Postoperative infectious complications correlated to the duration of warm ischemia.

cance or for the patient's ability to survive. The immunological significance was evident in cases in which the graft had to be removed because of irreversible rejection. An immunological reason could not be excluded in the case of transplantectomy due to acute late arterial bleeding from the grafted site. More debatable were the causes of patient death. Some of the deaths could be described as "non-immunological" patho-physiological courses, e.g. death after acute shock-provoking bleedings or after myocardial infarction. The important fact was however that a primary interference with immunological events could not be excluded. Even the most evident "non-immunologcial" death might very well be "immunological", i.e. due to unknown consequences of the immunoresponse. It was therefore considered exceedingly important not to exclude any patient from the material and not to have preconceived opinions about the causes of death.

Age, sex, cause and stage of uremia

There was an early and marked difference in graft and patient survival rate in the different age groups (Fig. 17, Fig. 27). It was notable that younger recipients had the lowest survival rate. This might be due to a different character of the original disease in younger patients or due to different immunological reactions of these patient. Kidney diseases rapidly leading to uremia in younger patients might be more malignant than slow-progressing diseases in older patients, concerning both immunological activity and direct life-threat.

The great differences in graft and patient survival of the two main diagnostic groups, pyelonephritis and glomerulonephritis was remarkable (Fig. 18, Fig. 28). Both of the diagnostic groups were heterogeneous but the poor results of transplantation of pyelonephritic patients have been noted earlier in our series. The difference in survival rate was not

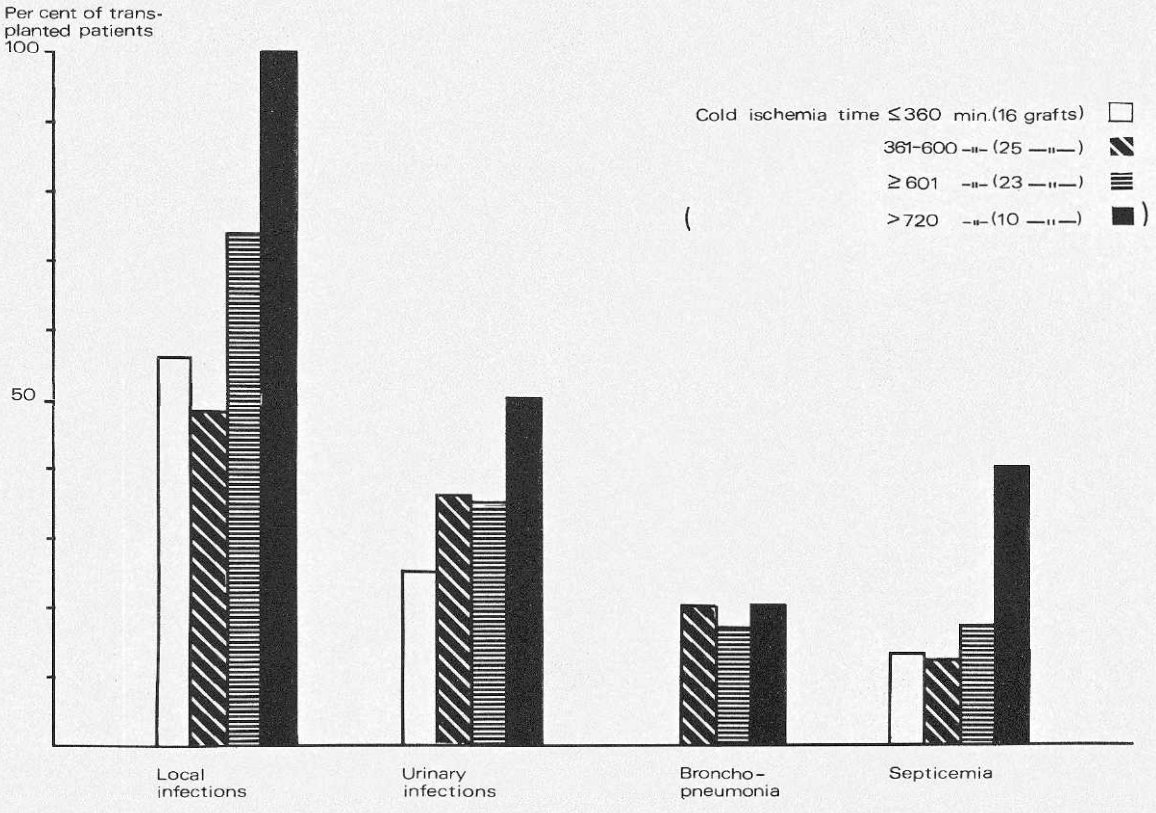

Fig. 35. Postoperative infectious complications correlated to the duration of cold ischemia.

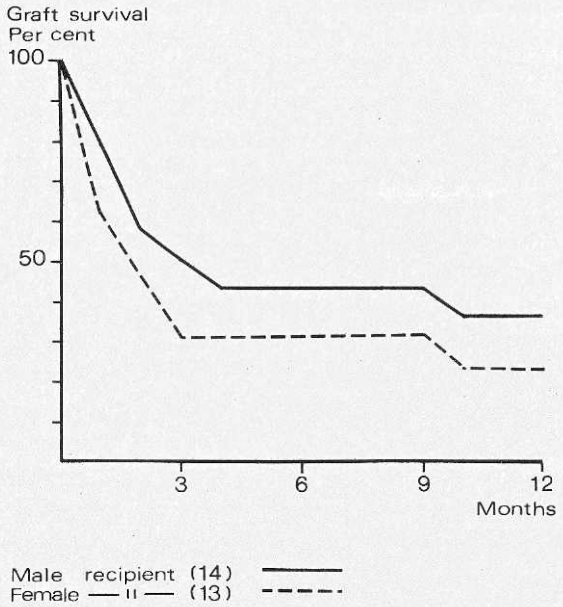

Fig. 36. Graft survival correlated to sex of the recipients with pyelonephritis.

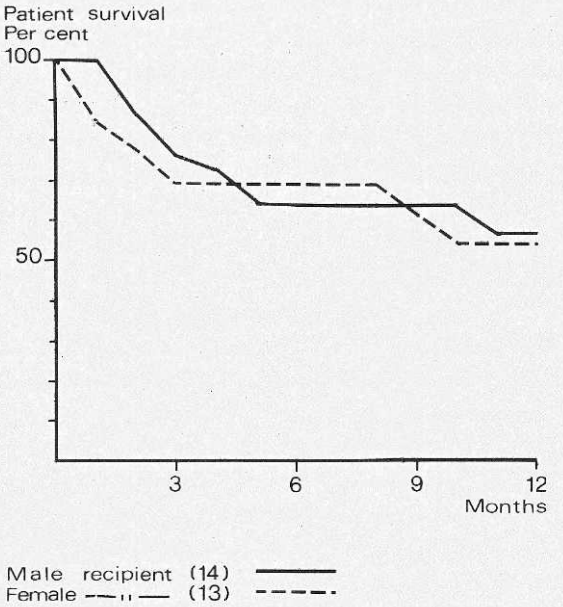

Fig. 37. Patient survival correlated to sex of the recipients with pyelonephritis.

due to the difference in sex distribution between patients with glomerulonephritis and pyelonephritis. The graft survival and patient survival was namely the same for males and females in the pyelonephritis group (Fig. 36, Fig. 37).

One possible explanation for this difference in survival rates was a difference in immunological events in recipients with glomerulonephritis and recipients with pyelonephritis. Pyelonephritis with urinary infections does affect the immunological defence mechanism of the patient. Antibodies developed against bacterial antigens might be able to "cross-react" against other antigens e.g. HL-A antigens or kidney antigens (Holm et al., 1972). Another possibility might be that patients with perpetual infections become immunological "strong" with a highly active immunoresponse mechanism.

Patients with polycystic kidney disease ought to be ideal transplantation candidates with slow-progressing uremia and ideal age. The results were however not better than for the glomerulonephritic patients. As urinary tract infections were fairly common in polycystic kidneys a certain resemblance to the

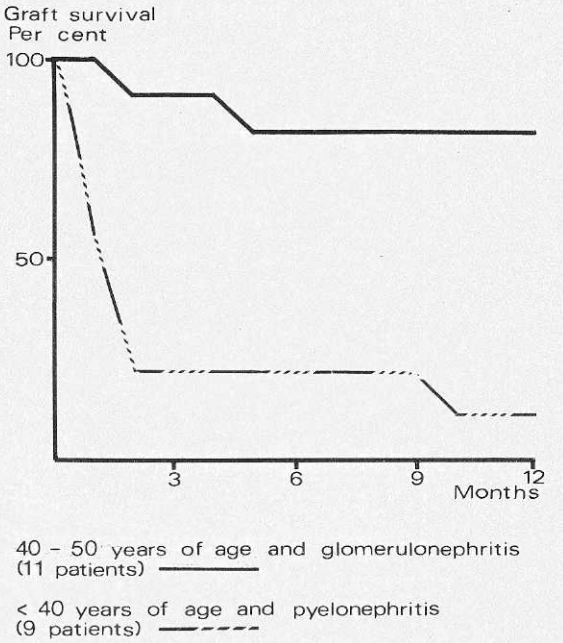

Fig. 38. Graft survival correlated to age and cause of uremia.

course of pyelonephritic patients might explain the results.

A finding supporting these theories of different immunological response capacity according to age and original disease of the recipient was the low graft survival rate of

younger recipients with pyelonephritis compared to the high graft survival rate of older recipients with glomerulonephritis (Fig. 38).

The duration of preoperative hemodialysis treatment had little influence on graft survival and no influence on patient survival (Fig. 19, Fig. 29). The higher graft survival rate in predialytic transplanted patients might be due to less risk for sensitization against HL-A or other antigens by less number of given blood transfusions. The higher graft survival rate in recipients dialysed for a long time might also have an immunological explanation. Patients on hemodialysis for a longer time receive more blood transfusions. Some of them then develop HL-A antibodies and their chances of obtaining a graft decreases; the preoperative cross-match with their serum and the potential donor lymphocytes will often be positive. Patients who do not develop HL-A antibodies despite multiple blood transfusions have thus a better chance of obtaining a kidney for transplantation. Because of their inability to develop HL-A antibodies they have been called "bad responsers", immunologically "weak" recipients with greater ability for graft acceptance (Opelz et al., 1972).

The different graft survival rates in patients with and in patients without their own kidneys remaining at the time of transplantation was found to be small and the patient survival was not influenced at all (Fig. 20, Fig. 30). The observed difference in graft survival could not be ascribed to the nephrectomy as this operation was performed only for specific indications. The graft survival curves may just indicate that these indications should be further limited.

Immunological aspects

An important factor for graft survival is the immunological competence of the recipient.

	No. of patients	Mean daily azathioprine dose Mg/kg
No proved incompatibility	21	2.4
One proved incompatibility	32	2.4
Most probably no incompatibility	14	2.4
Most probably one incompatibility	30	2.4
Most probably two incompatibilities	10	2.2

Tab. XX. Mean daily azathioprine dose during the first postoperative month correlated to the degree of compatibility.

	No.	Recipient dead with functioning graft	Transplantectomy due to rejection	bleeding
HL-A compatible graft	4	1	3	0
HL-A incompatible graft	6	2	1	3

Tab. XXI. Grafts lost within one month.

Age and original disease have already been mentioned as factors which probably influence this capacity. The problem of varying immunological competence of the recipients can also be expressed in another way. The fairly well standardized immunosuppressive regimen is sufficient for immunologically "weak" recipients but not for immunologically "strong" patients. Hence it is not only a question of immunological strength but a question of a counteraction of this power by the immunosuppressive therapy. In this series of transplantations all the patients received the same prednisolone dose. The same mean daily dose of azathioprine was given to recipients receiving HL-A compatible and HL-A incompatible grafts (Table XX). The variations in immunosuppressive therapy described earlier were dependent entirely on the individual bone-marrow depressing effect of azathioprine. As apparent from fig. 21 and fig. 31 a higher azathioprine dose resulted in a higher graft survival rate but a lower patient survival rate. These findings require further analysis.

Of the 64 grafts in this study 10 were lost within one month (Table XXI). The recipients of 23 of the remaining 54 grafts were given a lower azathioprine dose than that intended, 1.7 mg/kg/day, the other 31 a dose of 2.9 mg/kg/day. Ten of those 31 recipients (32%) were dead within one year (Table XXII). The mean daily dose for those ten patients was 3.0±0.0 mg/kg and until the beginning of the final complications they received an unchanged dose. All of them died with functioning grafts and all of them were transplanted with proved HL-A incompatible grafts. Of the 21 surviving recipients receiving the higher azathioprine dose three lost their grafts (one of eleven compatible, two of ten incompatible) within one year (Table XXIII).

Of the 23 recipients receiving the lower azathioprine dose four (17%) died within one year (Table XXII). All four were transplanted with proved HL-A incompatible grafts but only one patient died with a functioning graft; the other three died 4–7 months after transplantectomy due to irreversible rejection. Of the 19 surviving patients receiving the lower azathioprine dose eight lost their grafts (4/10 compatible, 4/9 incompatible) within one year (Table XXIII). These findings indicate that there was only a minor difference in survival rate of HL-A compatible and incompatible grafts provided the recipients received the same azathioprine dose and survived (Table

Mean daily azathioprine dose	No. of patients	Recipients dead within one year		
		Totally	With proved incompatible graft	With functioning graft
2.9 mg/kg	3	10	10	10
1.7 mg/kg	2	4	4	1

Tab. XXII. Mortality of 54 patients with grafts surviving > 1 month, correlated to azathioprine dose given during the first postoperative month.

Mean daily azathioprine dose	Graft survival >12 months		
	Totally	Of compatible grafts	Of proved incompatible grafts
2.9 mg/kg	18/21	10/11	8/10
1.7 mg/kg	11/19	6/10	5/9

Tab. XXIII. Graft survival in 40 patients surviving one year correlated to azathioprine dose given during the first postoperative month.

XXIV, Table XXV). There was however a difference in graft survival directly correlated to the given azathioprine dose independent of HL-A compatibility grade. The recipients receiving the higher azathioprine dose had a higher mortality than the recipients receiving the lower dose. The mortality occurred only among recipients receiving HL-A incompatible grafts.

Thus a high azathioprine dose was favourable for graft survival independent of compatibility grade but unfavourable for survival of recipients with HL-A incompatible grafts.

Histocompatibility

The most important single factor for graft survival in this series was found to be compatibility in the HL-A system. Compatibility did not imply absence of rejections; the frequency of transplantectomies due to irreversible rejections was the same as for incompatible grafts. The marked difference in graft survival rate was found to be related to the patient survival rate. Patients transplanted with incompatible grafts had a higher mortality rate and they often died with functioning grafts. Compatibility was also found to be the most important single factor for patient survival.

Of the 25 recipients transplanted with HL-A compatible grafts three died within one year, one of them with a functioning graft, two after transplantectomy. Of the 39 recipients trans-

	Mean azathioprine dose Mg/kg/day	No. of patients	One year patient survival rate Per cent	One year graft survival rate of surviving patients Per cent
No proved incompatibility (21 transplantations)	1.7	10	100	60
	2.9	11	100	91
One proved incompatibility (33 transplantations)	1.7	11*	82	55
	2.9	20	50	80

*After exclusion of two patients dead >7 months after transplantectomy (suicide, bleeding after retransplantation)

Tab. XXIV. Graft and patient survival correlated to proved compatibility grade and given azathioprine dose.

	Mean azathioprine dose Mg/kg/day	No. of patients	One year patient survival rate Per cent	One year graft survival rate of surviving patients Per cent
Most probably no incompatibility (14 transplantations)	1.7	5	100	80
	2.9	9	100	89
Most probably one incompatibility (30 transplantations)	1.7	12[1]	83	40
	2.9	17	59	80
Most probably two incompatibilities (10 transplantations)	1.7	4[2]	100	75
	2.9	5	40	100

1) After exclusion of one patient dead >7 months after transplantectomy (bleeding after retransplantation)
2) After exclusion of one patient dead >7 months after transplantectomy (suicide)

Tab. XXV. Graft and patient survival correlated to most probable compatibility grade and given azathioprine dose.

planted with proved HL-A incompatible grafts 18 died within one year, 13 with functioning primary grafts. These findings require a further analysis of the patient deaths, especially of deaths occurring despite functioning graft.

The causes of death were mainly infectious complications, thromboembolic or bleeding complications (Table XXVI). The incompatibility however did not lead to more frequent postoperative local complications (Fig. 39) as distinquished from prolonged ischemia times (Fig. 34, Fig. 35).

Most patients died later than one month after the transplantation. Four of them had clinical and histo-pathological signs of rejection during the final course (Table XXVI). It might be possible that transplantectomy could have saved their lives. None of these rejections was of the clinically irreversible type and they were probably provoked by an intentionally reduced immunosuppressive therapy. Thus, with standardized immunosuppressive therapy incompatibility in the HL-A system often led to death of the recipient despite functioning graft. The death occurred fairly late and was caused by infectious, thrombo-embolic or bleeding complications.

Main cause of death	Patient survival time Days	Signs of rejection at death
Septicemia	12	-
Septicemia	17	-
Septicemia	35	(+)
Septicemia	59	-
Septicemia	189	-
Cerebral hemorrhage	171	+
Cerebral hemorrhage	284	-
Hemopericardium (after open heart surgery)	130	-
Pancreatitis	54	-
Pancreatitis	83	+
Myocardial infarction	28	-
Heart failure	110	-
Pulmonary embolism	79	-
Acute rejection with multiple bleedings	57	+

Tab. XXVI. Main cause of death of 14 patients who died with functioning grafts.

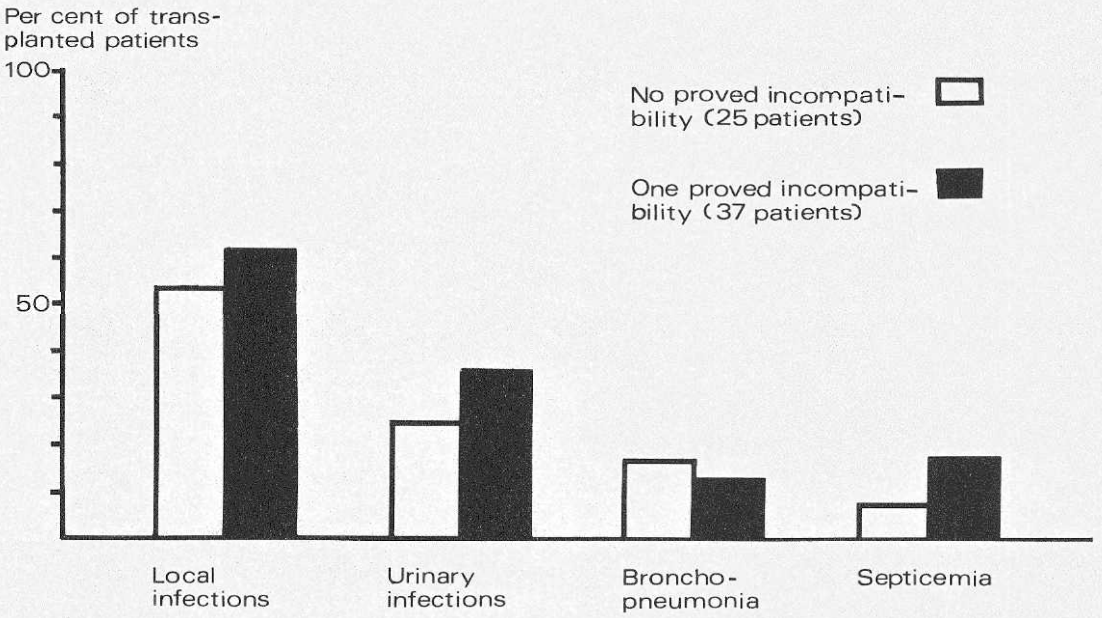

Fig. 39. Postoperative infectious complications correlated to proved incompatibility grade.

	Per cent of total material	Per cent of patients with one proved incompatibility	
		and graft survival >12 months (13 patients)	and graft survival <12 months (24 patients)
Age of recipient 40 - 50 years	41	46	29
Glomerulonephritis as cause of uremia	36	54	33
Transplanted predialytic	19	0	25
Transplanted after dialysis >12 months	11	23	12
Own kidneys left at transplantation	69	62	67
Mean daily azathioprine dose 2.9 mg/kg	57	62	54
Age of donor >50 years	30	46	25
Warm ischemia ≤40 minutes	89	93	83
Warm ischemia 31 - 40 minutes	22	38	12
Cold ischemia ≤600 minutes	64	77	63
Cold ischemia ≤360 minutes	25	31	25

Tab. XXVII. Frequencies of "positive factors" in transplantations of proved incompatible grafts, surviving or not surviving one year.

Loss of compatible kidneys

The graft survival was found to be superior after transplantation with HL-A compatible grafts (Fig. 22, Fig. 23). During the first 12 months however, nine of the 25 compatible grafts were lost. One patient died with a functioning graft due to septicemia 17 days after the transplantation. Seven grafts were removed because of irreversible rejection and one graft because of acute bleeding from the grafted site.

Of the 14 grafts with most probably no incompatibility two were removed because of irreversible rejection.

Thus HL-A compatibility between donor and recipient did not hinder irreversible graft rejection.

Long-term functioning incompatible kidneys

The graft survival rate was found to be lower after transplantation with HL-A incompatible grafts (Fig. 22, Fig. 23). One year after the transplantation however 13 of the 37 grafts with one proved incompatibility and 12 of the 36 grafts with most probably one incompatibility still were functioning.

One explanation for this might be an accumulation of "positive factors" among the functioning kidneys. Therefore the frequencies of earlier stated "positive factors" for graft survival were analysed in the group of transplantation with graft survival more than 12 months and compared to the same frequencies in transplantations with graft survival less than 12 months (Table XXVII, Table XXVIII). Most of the "positive factors" were found to be more frequent in transplantations with grafts surviving for more than 12 months, i.e. other factors favouring graft survival might have counteracted the disadvantages of HL-A incompatibility in the group of transplantations with graft surviving for more than 12 months.

Another possible explanation for the different course of transplantations with incompatible grafts might be a difference in severity of the proved HL-A incompatibility. Various HL-A antigens might be of various "strengths", "weaker" antigens more easy to

	Per cent of total material	Per cent of patients with most probable one incompatibility and graft survival >12 months (12 patients)	Per cent of patients with most probable one incompatibility and graft survival <12 months (24 patients)
Age of recipient 40 - 50 years	41	67	17
Glomerulonephritis as cause of uremia	36	67	33
Transplanted predialytic	19	0	25
Transplanted after dialysis >12 months	11	25	8
Own kidneys left at transplantation	69	67	62
Mean daily azathioprine dose 2.9 mg/kg	57	67	54
Age of donor >50 years	30	50	17
Warm ischemia ≤ 40 minutes	89	92	83
Warm ischemia 31 - 40 minutes	22	25	17
Cold ischemia ≤ 600 minutes	64	75	54
Cold ischemia ≤ 360 minutes	25	50	12

Tab. XXVIII. Frequencies of "positive factors" in transplantations of most probably incompatible grafts, surviving or not surviving one year.

transplant against (Hildeman, 1970). The incompatible HL-A antigen may also sometimes have a more or less pronounced "resemblance" to one of the recipients own antigens; such a relationship between certain HL-A antigens have been defined by the capacity of specific HL-A antibodies to react also against other specific HL-A antigens (Dausset & Hors, 1971, Kissmeyer-Nielsen et al., 1971, Thorsby et al., 1971b). An incompatibility with crossreacting antigens might imply a weaker antigen possibly inducing a weaker immune response in the recipient. To investigate if there were any such difference in "strength" of the different HL-A specificities in this series of transplantations performed against one specific proved HL-A incompatibility, the proved HL-A incompatibilities leading to graft loss within 12 months were compared to the proved HL-A incompatibilities leading to graft survival (Table XXIX). In addition the possibility of crossreacting antigens according to Thorsby were considered. It was found that very often the same specific HL-A antigen led to either graft

Incompatible HL-A antigen of graft surviving >12 months	of graft surviving <12 months	
	2*), 2	
9*)		
10		
11	11*), 11*), 11*)	First series (LA-locus)
28*)		
W19	W19	
7*)	7*), 7*), 7*), 7	
12, 12	12, 12	
W10*)	W10*)	
	W15*), W15	
W5, W5	W5	
27(AJ)*)	27(AJ)	
5(AJ)*)		Second series (4 locus)
	27*), 27	
	5	
	8, 8	
	13	
	W22*)	

*) Cross reactivity with recipient antigen possible

Tab. XXIX. Incompatible HL-A antigens of 13 grafts surviving one year and grafts not surviving one year.

loss or graft survival. There was a slight difference in graft survival if the incompatibility belonged to the first series (5/11 survived) or to the second series (8/26 survived). Possible cross-reactivity did not seem to improve the results.

One more aspect on the functioning incompatible grafts should be emphasized. As pointed out earlier the graft survival rate was higher if the recipient received a higher azathioprine dose independent of the compatibility grade. On the other hand the mortality rate also rose. Two conditions might then explain why HL-A incompatible grafts remained functioning:

1. The recipient received a lower azathioprine dose (and thus survived) which however for this special recipient meant a sufficient immunosuppression. The recipient might have been a "weak" responder or even more probable, the recipient was given a kidney with better compatibility in other relevant antigen systems outside the HL-A system.
2. The recipient was given a higher azathioprine dose and thus a more sufficient immunosuppression but survived despite the high azathioprine dose. The ideal condition with sufficient but not deleterious immunosuppression happened to occur.

Chapter 7

GENERAL DISCUSSION

The purpose of this study was to investigate the clinical importance of HL-A compatibility and incompatibility for the outcome of primary necrokidney transplantation. Two principles were considered to be of special importance for this evaluation, namely the demand for a uniform treatment of the grafts and of the recipients and the demand for avoidance of any selection of the patients.

Some defined factors of importance for graft survival were evaluated separately. Several factors were found to be of great importance but the most important single factor for graft survival was HL-A compatibility between donor and recipient. The graft survival rate was significantly higher when no HL-A incompatibility was proved and the graft survival rate was even higher when no HL-A incompatibility most probably existed, according to calculated chances for homozygotism of single antigens. The superior survival rate of compatible grafts was not due to a significantly lesser number and/or severity of rejection episodes but to a superior patient survival rate. The mortality was significantly higher if HL-A incompatibility existed. Most of these patients who died had functioning grafts. In fact loss of the recipient was the most common cause of loss of incompatible grafts. The causes of patient death were mainly infectious complications or conditions related to thrombo-embolic or bleeding complications, usually appearing fairly late after the transplantation. Among the investigated factors found to be of importance for patient survival, HL-A compatibility was the most important single factor. Again the significantly better patient survival with HL-A compatible grafts became even more evident when HL-A compatibility most probably existed.

Thus the HL-A compatibility between donor and recipient was found to be more important for patient survival than for graft survival.

This finding has not been pointed out earlier as all attention generally has been focused at graft survival. Many data from previous studies however support the correctness of this finding. Hill et al (1967) pointed out that the immediate postoperative mortality was diminished after some years of experiences while the late mortality, mainly due to infectious complications, seemed to remain unchanged. They also noticed that most of the patients who died late had functioning grafts. Moore and Hume (1969) stressed the fact that 80% of their transplanted patients who died had functioning grafts. When the importance of HL-A compatibility has been evaluated most authors have excluded patients who died with functioning grafts as "non-immunological" failures (Morris et al., 1970, Morris, 1971, Kissmeyer-Nielsen et al., 1971, Dausset & Hors, 1971, Batchelor et al., 1971, Amos, 1971). Also Terasaki and Mickey (1971) when discussing the problems of clinical evaluation of transplantation held the opinion that "non-immunological" deaths should be excluded. They regarded e.g. myocardial infarction as a "non-immunological" death but suicide as a complication to the transplantation procedure and thus necessary to include. The myocardial infarction might however very well

be due to an immune response mechanism in the recipient or to the immunosuppressive therapy, i.e. a real ''immunological'' death. A suicide, if performed long after graft failure and because of a hopeless dialysis situation might on the other hand be excluded as a ''non-immunological'' death.

It has thus been known that many transplanted patients die with functioning grafts late after the transplantation. In this series of transplantations such ''non-immunological'' deaths occurred almost exclusively among patients receiving HL-A incompatible grafts. Thus the findings in this study made it evident that HL-A compatibility was the most important single factor for graft survival because of *the influence of HL-A incompatibility on the recipient.*

How could incompatible HL-A antigens be fatal for the recipients and why was an incompatible antigen not always fatal? Three different mechanisms should be considered here:

1) The foreign HL-A antigen or its split products might have a direct toxic effect. Different antigens might be more or less toxic. As the chemical structures and properties of the HL-A antigens are still poorly known the possibility of a direct toxic effect of certain HL-A antigens cannot be dismissed. On the other hand no finding in this series or in other investigations support the suspicion of certain direct toxic HL-A antigens. An incompatible HL-A antigen might however constitute or raise a potential toxic substance lethal for the recipient only when some other certain conditions exist in the recipient, i.e. the antigen might induce a kind of graft-versus-host reaction.

2) The recipient's response to the introduced foreign HL-A antigen might induce a lethal disease. The immune defence might damage the leukocytes and thrombocytes of the recipients making them incapable of functioning adequately, i.e. a kind of auto-immune disease. The idea of an incompatibility induced auto-immune disease must be considered. If the fatal courses were consequences of too ''strong'' or ''broad'' immune response of the recipient they should have occurred more often among patients receiving less immunosuppression. On the contrary all but one of the recipients who died with functioning graft received the higher, intended immunosuppressive dose.

3) The introduction of a foreign HL-A antigen might change the basic conditions for the recipient's tolerance to the medical treatment. This raised the question if the incompatibility influenced the effect of the immunosuppressive therapy and must be discussed further.

The patients who died with functioning grafts died fairly late after the transplantation due to infectious, thrombo-embolic or bleeding complications. This clinical course is characteristic for too hard immunosuppressed patients. Such patients, who die with functioning grafts because of too strong immunosuppressive treatment are found in every reported transplantation series. As already mentioned these patients usually have been excluded as ''non-immunological'' failures. In this series of transplantations the immunosuppressive therapy was well standardized and all patients received the same treatment. The only existing divergency was a *lower* azathioprine dose given to 23 recipients who did not tolerate the intended dose because of bone-marrow depression. A lower azathioprine dose, although it was caused by a bone-marrow depression, rather seemed to protect the recipient from a fatal course. All patients but one, who died with functioning graft, received the higher, intended azathioprine dose. Of the patients who received the intended azathioprine dose no recipient with HL-A compatible graft died while 10 of 20 recipients of proved HL-A incompatible grafts died with functioning grafts.

One possible explanation for this will be discussed. The introduction of a new incompatible HL-A antigen into an individual stimulates

by definition a large proportion of the leukocyte population, larger than other antigens outside the HL-A system. The metabolites of azathioprine are antimetabolites which at the enzyme level interferes with the purine metabolism of cells. They have mainly a toxic effect upon cell division (Brent & Medawar, 1966). The total effect of the azathioprine depends not only on the concentration of its active metabolites (Nathan et al., 1961, Tinberger, 1968, Hitchings & Elion, 1969) but also on the number of dividing, i.e. stimulated cells. HL-A *compatible* grafts stimulate a certain portion of the leukocyte population. The division of the stimulated cells is counteracted by the azathioprine, more effectively the higher the azathioprine concentration. HL-A *incompatible* grafts stimulate the same proportion of the leukocyte population but moreover a much broader, "anti-HL-A-population" of leukocytes. The higher the azathioprine concentration is the better is the immunosuppressive effect but also the better is the suppression of the "anti-HL-A-population". This broader "not-only-graft-specific" immunosuppression might be deleterious for the patient if another cross-reacting antigen e.g. a bacterial or viral one, is introduced later. The portion of leukocytes stimulated by a second antigen might be a portion already stimulated by the foreign HL-A antigen and therefore damaged by the prolonged azathioprine therapy. This could explain the high incidence of deaths from infectious complications among recipients of HL-A incompatible grafts.

The divergent opinions of the correlations between HL-A compatibility grade and graft survival might then be explained by the different immunosuppressive treatment used in different transplantation centres. The deleterious HL-A-incompatibility-induced effect of azathioprine was only found when the higher dose was given. The immunosuppressive regimen is rarely described when the effect of HL-A compatibility is evaluated. It is known however that many transplantation centres are using lower azathioprine doses than in this series. Moreover antilymphocyte globulin was introduced as a new immunosuppressive agent almost simultaneously with the introduction of tissue-typing and was used routinely in many major transplantation centres. The addition of a new immunosuppressive drug often implied a reduction of other immunosuppressive drugs. Starzl et al (1968) e.g. decreased the mean daily azathioprine dose from 3.0 mg/kg to 1.9 mg/kg. Another immunosuppressive regimen might thus conceal the disadvantages of HL-A incompatibility by the simultaneous reduction of the azathioprine dose.

How then did the compatibility grade influence the graft survival in cases in which the recipient did not die despite this azathioprine sensitization? It is a well-known fact, often pointed out in the literature, that HL-A compatible grafts sometimes are rejected and HL-A incompatible grafts sometimes well accepted. In this series of transplantations irreversible rejections occurred with almost the same frequency in compatible and incompatible grafts (Table XVII and Table XVIII). In fact the size of the given azathioprine dose was more important for graft survival than the HL-A compatibility grade (Table XXVII and Table XXVIII). Furthermore, irreversible rejections did occur in HL-A compatible grafts even when the higher azathioprine dose was given and HL-A incompatible grafts were accepted even when the lower azathioprine dose was given.

The data obtained from this study indicate that HL-A compatibility was of minor or no importance for graft survival in the immunosuppressed recipients of primary necrokidney grafts. HL-A compatibility was however of great importance for the recipients' ability to survive an immunosuppressively effective intended azathioprine treatment.

Chapter 8

CONCLUSIONS

1. In 64 consecutive primary necrokidney transplantations compatibility in the HL-A system was found to be the most important single factor for overall graft survival during the first postoperative year.
2. This better graft survival was not due to a lower frequency of irreversible rejections but to a superior patient survival.
3. The degree of HL-A compatibility did not influence the survival of grafts in surviving patients.
4. The most important single factor for patient survival was HL-A compatibility between donor and recipient.
5. Patients transplanted with HL-A incompatible grafts who died had most often functioning grafts at the time of death. They had received an intended mean daily azathioprine dose during the first postoperative month of 2.9 mg/kg.
6. Patients transplanted with HL-A incompatible grafts and receiving a lower azathioprine dose survived.
7. Patients transplanted with HL-A compatible grafts survived independent of the size of the azathioprine dose given.
8. HL-A incompatibility was suggested to increase the risk for lethal toxic effects of azathioprine in the recipient.

Compatibility in the HL-A system between donor and recipient was of minor importance for graft survival during the first year after primary necrokidney transplantation. It was however of great importance for the recipients' ability to survive an immunosuppressively more effective azathioprine therapy.

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