A practical guide to granulocyte transfusion therapy

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A major development for the supportive care of patients with bone marrow suppression has been the recent introduction of techniques for collecting granulocytes from suitable donors. There is increasing awareness in this country that granulocyte transfusion for infected neutropenic patients is now a practical possibility, and the demand for granulocytes from those centres able to provide this service has increased considerably. For example, the number of transfusions provided by the Royal Marsden Hospital in the year 1972/73 was 19; the figures for the following two years were 115 and 116. Equipment is now available which is cheaper and simpler to operate than the earlier machines, and this means that granulocyte transfusions can now be provided by centres unable to afford the expertise and equipment previously required. It seems therefore an appropriate time to give some account of the available methods of collecting granulocytes, the evidence for their value, and the indications for their use.

The Need for Granulocyte Transfusions

Since the introduction of routine platelet transfusions, infection has become the major cause of death in patients with bone marrow suppression (Levine *et al*, 1972). While germ-free environments and oral non-absorbable antibiotic regimes have done much to reduce the acquisition of infection in hospital (Jameson *et al*, 1971; Levine *et al*, 1973; Yates and Holland, 1973; Schimpff *et al*, 1975), infections still occur despite these measures, and many neutropenic patients are already infected when admitted to hospital. The effectiveness of many of the most useful antibiotics is reduced in the presence of neutropenia (Levine *et al*, 1972), and antibiotic resistance may well become more of a problem than it is at present (Greene *et al*, 1973). Attempting to reverse the fundamental defect of neutropenia by transfusion of granulocytes is a logical approach to this problem but it is complicated by the fact that only a small proportion of the total granulocyte pool of a normal individual is present in the circulation,

and the mature granulocyte is a relatively short-lived cell. Thus a normal adult has approximately 1.5 to 3×10^{10} circulating granulocytes which represents only 1-2% of the total granulocyte pool. The estimated daily turnover is about 1.2×10^{11} cells, and the mature neutrophil spends on average only 10 hours in the circulation (Athens *et al*, 1961; Boggs, 1974). Using these figures, it can be seen that it would require the transfusion of the granulocytes from 40 to 80 units of donor blood simply to replace the normal daily requirement without compensating for the increased consumption of granulocytes due to infection. While it may not be necessary to give quite such large numbers of cells to obtain a therapeutic effect, some method of concentrating granulocytes from normal blood is clearly essential before the transfusion of useful numbers of these cells can be contemplated. An alternative is to use cells from patients with chronic granulocytic leukaemia (CGL) where there is often no need for concentration because of the high peripheral blood count.

Collection of Granulocyte Concentrates

The methods of collecting granulocyte concentrates depend on the circulation of donor blood through a device which selectively removes the granulocytes and returns processed blood into the opposite arm of the donor. The selective removal of granulocytes is performed by either a specially designed centrifuge bowl or a nylon fibre filter.

CENTRIFUGAL CELL SEPARATORS

In these machines anticoagulated donor blood passes into a centrifuge bowl where separation takes place into red cells and plasma between which is a layer of leucocytes. This layer can be removed into a bag and recombined red cells and plasma are returned to the donor. Two kinds of machine are available, depending on whether the filling of the bowl and the return of processed blood to the donor is continuous or discontinuous.

In the continuous-flow machines (IBM¹ and

Aminco¹), processed blood is removed from the bowl and returned to the donor continuously so the only change in blood volume is due to the slow continuous loss of buffy coat which should not normally exceed 500 ml. In these machines the centrifuge bowl is made of polycarbonate or stainless steel and requires sterilization, but all plastic tubing is disposable².

The second kind of machine (Haemonetics Model 30³) operates on a discontinuous separation principle so that filling of the bowl and return of blood to the donor are separate processes. After granulocytes and platelets have been collected red cells and plasma removed from the bowl have to be returned to a transfusion bag whence they are reinfused into the donor under gravity while the subsequent filling of the bowl takes place. This means that the volume of blood outside the donor at any one time tends to be greater than with continuous-flow machines and the changes in blood volume are intermittent. Because both bowl and tubing for this machine are disposable it is relatively simple to operate. It is necessary to use acid-citrate-dextrose (ACD⁴) as anticoagulant with this machine whereas heparin, which causes fewer side effects, can be used with the continuous-flow separators.

Other comparative merits of these machines are discussed in more detail below and illustrated in the table.

FILTRATION LEUKAPHERESIS

This technique, first described by Djerassi *et al* (1970), depends on the ability of granulocytes to adhere to nylon fibres from which they can subsequently be eluted. The procedure will be described in a little more detail because its simplicity means that it may possibly become much more widely used than the cell centrifuges.

The simplest form of apparatus required is illustrated (fig 1). Here the only mechanical device is a simple rotary pump. The filters consist of plastic cylinders filled with nylon fibres⁵. These filters and the connecting plastic tubing are primed with heparinized saline and all air bubbles are excluded from the system. After cannulation of both arms and connection of the cannulae to the circuit the donor is given a loading dose of 3000 units of heparin intravenously. While perfusion takes place hepari-

nized saline is added at a rate of 5000 units per hour into the blood flowing out of one antecubital vein. This blood then passes through a device for monitoring the flow which can be an electronic pressure sensor⁶ or a simple plastic chamber which collapses when flow from the vein is inadequate. The rotary pumps should preferably be placed before the filters because they occasionally collapse if blood is drawn through them, leading to poor granulocyte recovery. If more than one filter is being perfused, as is generally the case, separate or dual-channelled pumps must be used so that the volume flowing through each filter is known. The return of filtered blood to the donor must involve some mechanism to prevent air being pumped back. This can be achieved either by pumping blood into a re-infusion bag whence it is returned by gravity (fig 1) or by passing it through a bubble trap which activates a warning device and switches off the pumps automatically if air bubbles are present⁶. As bleeding and re-infusion are a continuous process, the total volume outside the donor need not exceed 300 ml at any time. Because of variability in the number of granulocytes needed to saturate a single filter and variation of donor blood counts before and during leukapheresis, it is impossible to know exactly how much blood to perfuse on a single occasion. However, satisfactory yields are usually obtained by passing about 3 litres of blood through each filter. If two filters are used this usually gives a running time of 2 to 3 hours which is well tolerated by most donors. At the end of the procedure the small volume of red cells and plasma remaining in the circuit is flushed back to the donor with saline and the granulocytes are eluted from the nylon fibres by passing a litre of ACD/plasma/saline pH 6.5 through each filter. The granulocyte suspension is then concentrated by centrifugation at 1000 *g* for 15 minutes and infused directly into the recipient.

Collection of Granulocytes from Normal Donors

CELL YIELDS

The number of granulocytes collected on a single occasion (hereafter referred to as granulocyte yield) depends on the total volume of blood processed and the granulocyte count of the donor during collection. The available collection procedures vary in their efficiency, ie, in the proportion of the estimated total number of cells processed which can be retrieved.

The buffy coat collected by a centrifuge from a normal donor tends to have a lower proportion of

¹V. A. Howe and Co Ltd, 88 Peterborough Road, London SW6.

²Avon Medicals Ltd, Capon Heaton, Birmingham B30 3DR.

³Haemonetics Corporation, 368 Northolt Road, South Harrow, Middlesex HA2 8ES.

⁴A concentrated solution containing sodium citrate 29.33% w/v and citric acid 10.64% w/v can be made up by a hospital pharmacy. 30 ml of this solution is then added to 400 ml of sedimenting agent (see below).

⁵Leukopak (Fenwal Ltd), Travenol Laboratories, Thetford, Norfolk IP24 3SE.

⁶Such a device is fitted to continuous-flow separators and to the Fenwal leukapheresis pump.

Method	Centrifugation		Filtration ¹	
Machine	IBM + Aminco		Fenwal	Simple Pump
Purchase price (£) ²	23-26 000		1800	Negligible
Running cost per leukapheresis (£) ²	35-40 ^{3,4}		21	17
Ease of operation and maintenance	++		+++	+++
Donor comfort	++			
Efficiency of granulocyte collection	++ ⁵		+++	+++
Quality of granulocytes	+++		++	
Value for other functions				
Platelet collection	++		?*	
Plasma exchange	+++		—	
Collection of lymphocytes and blast cells	++		—	

Table Relative merits of the available methods of granulocyte collection

¹May also be carried out with the centrifugal machines at a cost of about £20 per run.²Prices can be only approximate because of inflation and fluctuating exchange rates.³Assumes the use of HES.⁴Includes an estimate based on the finite life of a centrifuge bowl.⁵There is no direct evidence concerning the quality of these cells but there is no reason to suspect that they will function differently from those collected by the continuous-flow machine unless the higher bowl speed has a damaging effect.^{*}The equivalent of several units of platelets may be collected by a single procedure but there is no direct evidence concerning the clinical effectiveness of these.

This table should be used as a rough guide only. Comparisons are made on a scale of + to +++ in order of increasing value. Some of the comparisons are necessarily subjective and are based on our own experience with these techniques.

Fig 1 Diagram of simple apparatus for filtration leukapheresis.

granulocytes than the peripheral blood because the relatively high density of the granulocyte tends to make it sediment into the red cell layer. This means that a centrifuge is not as efficient for collecting granulocytes as it is for mononuclear cells. The efficiency of separation may be increased by adding a red cell sedimenting agent such as hydroxyethyl

starch (HES)¹ to the donor blood. This has the effect of decreasing the trapping of granulocytes in the red cell layer. Mishler *et al* (1974) have reported an increase in mean granulocyte yield from 1.0×10^{10} to 4.7×10^{10} with the use of HES. Another way of increasing the number of cells collected is to use prednisolone or dexamethasone to increase the circulating granulocyte count in the donor. Using both sedimenting agents and steroids, Benbunan *et al* (1975) have achieved a mean yield of 5.2×10^{10} , although the yields obtained by two other groups of workers using similar methods are somewhat lower at 1.7×10^{10} (McCredie *et al*, 1975; Lowenthal *et al*, 1975).

The Haemonetics separator is not as efficient as the continuous-flow machines for collecting granulocytes. Sedimenting agents appear to be essential if useful numbers are to be obtained and it is logical to use steroids as well. Sussman and Colli (1975) have reported an average yield of 1.5×10^{10} using both agents, and Huestis *et al* (1975) obtained a mean yield of 1.3×10^{10} using a sedimenting agent without steroids.

Filtration is a more efficient way of collecting granulocytes than centrifugation. Once again steroids may be useful, and we were able to increase our mean yields from 2.0 to 3.2×10^{10} by premedicating donors with dexamethasone. Most other workers appear to

¹HES is supplied by McGraw Laboratories Division, American Hospital Supply Corp, Evanston, Illinois, USA. Alternative agents include Plasmagel and Dextran.

obtain average numbers of $3-4 \times 10^{10}$ cells per procedure (Russell and Powles, 1976). Djerassi *et al* (1975) have in fact been able to report mean yields ranging from 3 to 10×10^{10} cells in unstimulated donors.

SIDE EFFECTS

The most common complication experienced by donors is a sensation of light-headedness. Our experience is that this tends to be encountered somewhat more frequently with the Haemonetics separator perhaps because the changes in blood volume are greater with this machine. More severe vasovagal attacks may occur with any machine. Loss of consciousness in the supine position has been reported (Buchholz *et al*, 1975) and we have observed a similar episode. Shivering attacks may occur in a small percentage of donors (Buchholz *et al*, 1975) although we have not seen this complication in over a hundred donations using filtration leukapheresis. Repeated daily filtration leukapheresis of a single donor has been used without ill effect (Schiffer *et al*, 1975b). The use of ACD with the Haemonetics separator may produce nausea and paraesthesiae in some donors but on the whole the use of this machine appears to be well tolerated (Huestis *et al*, 1975; Szymanski *et al*, 1973). Complications of anticoagulation with heparin have not been reported and it does not seem to be necessary to reverse its effect with protamine. Likewise, we are not aware of any immediate hazards associated with the use of sedimenting agents or steroids in healthy donors.

While there is no evidence to date that these collection procedures present any short-term risks to donors, it is imperative that any procedure widely practised on normal individuals should be known to be as safe as possible. This fact has led some workers, ourselves included, to have some reservations concerning the use, in particular, of sedimenting agents. Anyone considering using these techniques should be aware of the ethical implications of infusing foreign substances into normal individuals where the long-term effects in particular are unpredictable.

DONOR SELECTION

Careful selection of donors may prevent unnecessary discomfort for some and possible danger to the occasional individual. We feel it is preferable to err on the side of caution and generally to restrict the age of donors to between 18 and 50 years. It is probable that donors over 50 would have a greater risk of cardiac arrhythmia or cerebrovascular accident, however slight. A general rule of this kind, which can be relaxed on occasion, ensures that extra care is taken if selection of an older donor is unavoidable because no one else is available. No donor

should have a history of a medical condition which would be a contraindication to anticoagulation or to routine blood donation. It is important to check that the veins are of adequate size in order to avoid subjecting the donor to a lengthy and perhaps fruitless procedure. It is useful to have a printed information sheet for donors to read and to require them to sign a consent form. Individuals who appear to be excessively anxious or who have a history of fainting should not be asked to undergo the procedure.

Donors should be ABO group compatible with the recipient and have a normal blood count. The use of close relatives makes a degree of histocompatibility more probable but is to be avoided if future bone marrow transplantation is contemplated (see below).

EFFECT ON DONOR BLOOD COUNTS

Both methods of leukapheresis produce changes, usually transient, in donor blood counts (Higby *et al*, 1975; Russell and Powles, 1976). A fall in haemoglobin level of about 10 g/l usually occurs, largely due to haemodilution by saline. The granulocyte count may show a profound fall during the first 30 minutes of filtration leukapheresis (Schiffer *et al*, 1975a), but at the end of both procedures about 80% of donors will show an increased count, even without the use of steroids. The filtration method tends to remove more platelets than the centrifuge and may reduce the count to less than 50% of the initial level. Although the haemoglobin and platelets usually return to their initial levels very quickly (Higby *et al*, 1975), a more prolonged effect is occasionally seen (Russell and Powles, 1976).

Use of Donors with Chronic Granulocytic Leukaemia

Patients with CGL are a valuable source of granulocytes for transfusion (Morse *et al*, 1966; Mathé *et al*, 1971). With high blood counts it is possible to use whole fresh blood or to concentrate the granulocytes after sedimenting the red cells which can then be reinfused into the donor. If a centrifugal separator is used, numbers of granulocytes up to 10^{12} can be removed in exchange for whole blood or plasma, and this may have some therapeutic value for the donor. Where the count is lower, between about 20 and $100 \times 10^9/l$, the centrifuge will provide useful yields which can be improved with the use of sedimenting agents. With these lower counts it is important that the donor should be fully aware that the procedure will be of little direct benefit to him.

CGL is not a common disease and it is often difficult to find a suitable donor. A register of such patients has been set up in the London area in an attempt to get over this problem, and this principle could be extended to other parts of the country.

Behaviour of Infused Granulocytes

The behaviour of granulocytes transfused into allogeneic recipients is complex (Boggs, 1974), and care is needed in interpreting the results of any *in vitro* and *in vivo* studies. Examination of structure and function *in vitro* may be useful to assess damage to the cells by the collection procedures, but the ability of such tests to predict behaviour *in vivo* may be limited. Thus a cell may be structurally and functionally intact on *in vitro* testing but still unable to perform a useful function *in vivo* because of factors such as antibodies operating in the recipient. Some assessment of activity *in vivo* may be provided by isotopic labelling of cells and counting the radioactivity of an infected area, or by detecting migration to skin windows. However, even these tests can indicate activity in only a proportion of infused cells. The increment in granulocyte count, commonly measured one hour after infusion, is similarly difficult to interpret. Although there is no clear relationship between such increments and clinical response, the fact that small increments tend to be associated with factors which might be expected to give a poor response, such as antibodies, apparently damaged cells, and splenic sequestration (Eyre *et al*, 1970; Herzig *et al*, 1972), suggests that this measurement may have some value. On the other hand, a low increment may not necessarily mean that the transfusion is of little value in a given case as it may simply indicate a more profound deficit in total granulocyte pool or increased utilization due to infection (Morse *et al*, 1966; Boggs, 1974). Clearly, the only satisfactory answer concerning the value of granulocyte transfusion can be provided by clinical response. On an individual basis response can be assessed by lysis of fever, resolution of infected lesions or survival for a given length of time. However impressive anecdotal experience may be, the most satisfying evidence is that provided by controlled clinical trials. These have understandably been few in number and are liable to become more difficult to perform ethically as evidence for the value of this form of treatment accumulates.

With these qualifications in mind some of the evidence for the value of transfused normal and CGL cells will be briefly reviewed.

NORMAL CELLS

There is some evidence that the collection of granulocytes by filtration damages the cells to some extent. Herzig *et al* (1972) reported that light and electron microscopy revealed cytoplasmic vacuolation and a ragged appearance to the cell membrane in 15-20% of filtered cells. While such changes need not necessarily imply functional impairment (Schiffer

et al, 1975b) the function of filtered cells as measured by phagocytic and bactericidal tests may be impaired to some extent (Herzig *et al*, 1972; Higby *et al*, 1975.) Such changes do seem to be at least partially reversible by restoring the cells to a more physiological medium (Debelak *et al*, 1974; Sanel *et al*, 1975). *In vitro* bactericidal and phagocytic tests appear to be performed normally by cells collected by the centrifuge (Mishler *et al*, 1974). *In vivo*, post transfusion increments corrected for dose are lower with filtered cells than with those collected by the centrifuge (Graw *et al*, 1972; Schiffer *et al*, 1975b), and experiments in dogs have shown that considerable numbers of filtered cells are rapidly removed by the spleen (Herzig *et al*, 1972).

It appears, therefore, that an equivalent dose of cells collected by the centrifuge is likely to be more effective. Nevertheless, there is good evidence that filtered granulocytes can perform some functions *in vivo*. Debelak *et al* (1974) rendered dogs neutropenic by irradiation and were able to protect them from septicaemic episodes by using filtered granulocytes. Skin window experiments in man have shown that filtered cells can migrate to sites of infection (Schiffer *et al*, 1975b), and appreciable numbers of these cells may occasionally be seen to circulate in the recipient for many hours (fig 2).

In 1972 Graw *et al* reported a controlled trial in which they concluded that normal cells collected by both methods were of value, particularly if four or more daily transfusions were given. Thus all 12 patients receiving four or more consecutive daily

Fig 2 Case 1. Response of temperature and granulocyte count to three granulocyte transfusions.

granulocyte transfusions had complete recovery compared with 5 of 19 patients in their appropriate control group (Graw *et al*, 1972). More recently, the clinical effectiveness of filtered granulocytes has been confirmed by Higby *et al* (1975). In their study only 5 of 19 control patients survived to day 20, compared with 15 of 17 patients who received four consecutive daily transfusions.

The following case history describes a situation in which the transfusion of filtered granulocytes appears to have been useful.

Case 1

T. C., a 5-year-old girl, was admitted to hospital with epistaxis following chemotherapy for acute lymphoblastic leukaemia. On admission the haemoglobin level was 60 g/l, platelet count $5 \times 10^9/l$, and white count $1.9 \times 10^9/l$ with 1% neutrophils. She became febrile and treatment with ampicillin was begun. On the third day she was transferred to the Royal Marsden Hospital for supportive care. At this stage she had clinical and radiological evidence of a chest infection, and gentamicin and carbenicillin were added to the antibiotic regime. Figure 2 shows the subsequent course of her temperature and granulocyte counts. Her condition initially deteriorated so that 48 hours after transfer she was cyanosed, in an oxygen tent, and critically ill. After an infusion of 2×10^{10} granulocytes collected by filtration leukapheresis from her father her temperature fell and her condition began to improve. The following morning, 16 hours after the transfusion, her granulocyte count was $1.33 \times 10^9/l$. A second transfusion of 1.8×10^{10} cells from an aunt was given 24 hours after the first, and the count the following morning had increased to $2.27 \times 10^9/l$. Although the temperature remained normal and her clinical condition improved she was given another transfusion of 1.9×10^{10} cells from her father 48 hours after the second. The granulocyte count, which had fallen to $0.23 \times 10^9/l$ before the transfusion, rose to $1.98 \times 10^9/l$ at 16 hours and then fell again 24 hours later to $0.23 \times 10^9/l$. The patient continued to improve and her granulocyte count showed spontaneous recovery, reaching $1.0 \times 10^9/l$ eight days later. She was discharged from hospital four weeks after admission and remains well and in remission nine months later.

This case history illustrates a number of points. The improvement in the temperature and clinical condition seems to have been related to the granulocyte transfusion. Moreover, the increased granulocyte counts observed 16 hours after transfusion are unlikely to have been due to spontaneous recovery because the levels fell again after a further 24 hours in the absence of further transfusion and

the eventual spontaneous recovery took over a week to produce comparable figures. The fact that the transfused cells could persist for so long in the circulation is encouraging and may be related both to the relatively high dose which could be given to a child and also to a degree of histocompatibility achieved by using relatives as donors (see below).

Reactions in Recipients of Normal Cells

Transfusion reactions to normal granulocytes seem to occur more often when filtered cells are used. This could possibly reflect the greater degree of cell damage in these preparations (Schiffer *et al*, 1975b). A small increase in temperature accompanied by shivering has been reported in up to 60% of recipients of these cells (Higby *et al*, 1975). More severe symptoms, which may include respiratory difficulty and hypotension, have occurred in a significant number of patients. Thus Schiffer *et al* (1975b) reported reactions classified as 'moderately severe' in more than 25% of their patients. There appears to be no clear relationship between such reactions and HLA incompatibility or detectable leucocyte antibodies (Schiffer *et al*, 1975b). These reactions may be controlled by stopping the transfusion and giving antipyretics, antihistamines, and corticosteroids. It may in fact be possible to avert them altogether by giving the cells slowly (about 1×10^{10} per hour) and premedicating the recipient with hydrocortisone and antihistamines (Djerassi *et al*, 1975).

CGL CELLS

Although *in vitro* tests of the bactericidal and phagocytic properties of CGL cells sometimes show that they do not function quite as well as normal granulocytes (Penny and Galton, 1966), any defect there may be is probably compensated for by the large numbers of these cells which are available. *In vivo* studies have shown that such cells have the capacity to ingest organisms and to migrate to inflammatory lesions (Eyre *et al*, 1970; Shohet, 1968). Transfusion of large numbers of CGL cells can produce substantial increments in leucocyte counts in the recipient. For example, Morse *et al* (1966) found a median increase in circulating granulocyte count of $1 \times 10^9/l$ one hour after transfusion. In addition, younger cells may mature in the recipient and occasionally a temporary haemopoietic graft is produced with prolongation of the therapeutic effect (Buckner *et al*, 1969; Mathé *et al*, 1971). Although their clinical studies were not controlled, many investigators have concluded that CGL cells are of considerable value in the control of infection (Eyre *et al*, 1970; Mathé *et al*, 1971; Lowenthal *et al*, 1975). Indeed, it is difficult to avoid the impression that a large number of CGL cells may

succeed in some cases where normal cells are inadequate. This is illustrated in the following case history.

Case 2

A 7-year-old girl with aplastic anaemia developed a chest infection which was treated with gentamicin, cloxacillin, and carbenicillin starting on 18 April 1974. In addition she was given daily transfusions of granulocytes collected by filtration from unrelated donors (fig 3). After a week of treatment her condition had deteriorated and she was given an infusion of 2.1×10^{10} irradiated CGL cells without effect. She was then critically ill and on 20 April was given 1.2×10^{10} non-irradiated cells from the same donor. Her condition improved dramatically and a second dose of 2.1×10^{10} non-irradiated cells was given three days later. Bone marrow specimens on 1 and 3 May were consistent with CGL, and dividing cells were shown to contain the Philadelphia chromosome. Two further transfusions of irradiated cells were given on 2 and 6 May. The peripheral granulocyte count, which included many immature forms and had risen to over $2 \times 10^9/l$ by 30 April, remained between 1 and $4 \times 10^9/l$ for the following eight days. On 10 May she began treatment with cyclophosphamide, 1.25 g daily for four days, and a bone marrow transplant from her sister was performed on 15 May. Subsequent blood counts were complicated by the fact that she then received daily transfusions of irradiated cells from a patient with a myeloproliferative syndrome, but by 28 May the marrow showed a virtual absence of haemopoietic cells. The attempted

graft failed to take, and a second attempt a month later was also a failure.

The first lesson to be learnt from this case concerns the failure of daily transfusions of filtered granulocytes to control the infection. Although the yields from several donors were somewhat unsatisfactory, daily transfusions of 0.34 to 3.5×10^{10} cells might have been expected to produce some improvement in a child of this age. Failure can possibly be attributed to a combination of severe infection, HLA incompatibility, and profound neutropenia. Even a dose of 2.1×10^{10} irradiated CGL cells produced no response, and it was only after a somewhat lower dose of non-irradiated cells were given that dramatic improvement was seen. Further irradiated cells were not given until the patient was very much better. Evidence for a haemopoietic graft was provided by the well-maintained neutrophil and platelet counts and the presence of dividing Ph^1 positive cells in the bone marrow. The rationale for irradiating granulocyte transfusions, and withholding the irradiation on occasion, is discussed below.

Complications of Transfusion of CGL Cells

Transfusion reactions may occur as with normal granulocytes and they tend to be more severe, possibly due to the larger numbers usually given. An association of such reactions with the presence of leucocyte antibodies has been reported (Goldstein *et al*, 1971). Particularly alarming reactions, characterized by respiratory distress and cyanosis, may occur and have been attributed to the sequestration

Fig 3 Case 2. Temperature and neutrophil counts. All transfusions were irradiated with 1500 r except where indicated.

of large numbers of cells in the pulmonary capillary bed (Mathé *et al*, 1971).

The development of grafts of CGL persisting for several weeks has occasionally been reported (Lowenthal *et al*, 1975; Coltman *et al*, 1975). In addition, graft versus host (GVH) disease may occur in immunosuppressed patients (Graw *et al*, 1970; Mathé *et al*, 1971).

Irradiation of Granulocytes

The decision to irradiate a granulocyte suspension depends on the source of the cells and the clinical state of the recipient. Cells from normal donors are irradiated in order to prevent division of lymphoid cells and the development of GVH disease in immunosuppressed individuals. A dose of 1500 rad is commonly used. This figure is based on the dose required to abolish the response of lymphocytes in mixed lymphocyte culture and apparently does not alter normal granulocyte function (Graw *et al*, 1970). We are not aware of any reports of GVH disease following irradiation of granulocyte suspensions with this dose. As the development of GVH disease probably depends on the dose of lymphocytes infused it must be remembered that cells collected by the centrifuge contain a relatively high number of lymphocytes whereas filtered preparations usually contain less than 5%. However, we feel it is safest routinely to irradiate all blood products where there is severe immune depression.

In the case of CGL cells irradiation will inhibit not only division of lymphoid cells but also that of stem cells which might otherwise be responsible for producing a haemopoietic graft. There is some evidence that irradiation of CGL cells may impair their effectiveness (Bussel *et al*, 1975), and the establishment of a temporary graft may be beneficial, as in our case 2 above. In addition, such a graft may exert a temporary antileukaemic effect which is related to GVH disease (Mathé *et al*, 1971). These potential benefits must be weighed against the risk of obtaining a persistent graft with or without a potentially fatal GVH reaction. We feel that there should be no hesitation in using non-irradiated CGL cells in situations where there has been no response to irradiated cells and it is felt that the infection will otherwise prove fatal.

Donor Recipient Matching

Persistence of transfused granulocytes in the circulation has been shown to decrease with increasing ABO and HLA incompatibility between donor and recipient and the presence of leucocyte antibodies in the recipient (Goldstein *et al*, 1971; Graw *et al*, 1972).

This raises the possibility that these factors may also impair clinical effectiveness but at present there is no direct evidence that this is the case. Unfortunately time does not always permit *in vitro* tests other than ABO grouping but the use of relatives as donors often leads to some degree of histocompatibility. However, the use of related donors should be avoided in situations where bone marrow transplantation is contemplated because the graft is less likely to take if blood products from relatives have previously been given (Thomas *et al*, 1975).

Indications for Granulocyte Transfusion

While any infection in which neutropenia is a significant factor might be expected to benefit from granulocyte transfusion, the limited facilities usually available means that in practice only a small minority of patients are able to receive such support. The decision to provide support depends on a balanced assessment of the clinical situation with particular reference to the nature of the underlying disease and the estimated chance of controlling the infection with antibiotics alone. In a situation where most patients treated in this way are suffering from a malignant disease it may be justifiable to restrict the provision of granulocyte transfusion to those patients in whom it is felt that a remission of the disease can be achieved. It does in fact appear to be more difficult to control infection in cases where the underlying disease is not responding to chemotherapy (Smith *et al*, 1975). Because many infections appear to be controllable by antibiotic therapy alone, even in the presence of severe neutropenia, it seems reasonable to try the effect of antibiotics alone for 48 hours from the onset of the infection. If the infection is already advanced when first seen, granulocyte support should be considered from the outset if the cells are readily available. Although infection in the presence of a neutrophil count of less than $0.5 \times 10^9/l$ is often taken as an indication for granulocyte transfusion (Graw *et al*, 1972; Higby *et al*, 1975) our own experience is that such transfusions are rarely requested until the count has fallen below $0.2 \times 10^9/l$. This would in fact appear to agree with the experience of Higby *et al* (1975). Although they stated that a count of less than $0.5 \times 10^9/l$ qualified an infected patient for entry into their trial, their data reveal that all 36 of their patients had granulocyte counts of $0.2 \times 10^9/l$ or less at the beginning of the study.

HOW MANY CELLS SHOULD BE GIVEN?

Several investigators have reported a clear relationship between response to transfusion and both the numbers of cells infused and the number of trans-

fusions given (Morse *et al*, 1966; Graw *et al*, 1972; Lowenthal *et al*, 1975). In their controlled trial, Graw *et al* found that the difference in outcome of infection between their control and treated groups reached statistical significance only when four consecutive daily transfusions were given. It seems that the decision to give granulocytes should involve being prepared to give daily transfusions for at least four days. Clearly, the more cells given per transfusion the more useful are they likely to be. The daily doses of cells given by most groups of workers generally reflect the number of cells which can reasonably be removed from one donor in a single procedure. Analysis of the cell numbers given by investigators who have claimed some benefit from granulocyte transfusion suggests that one should aim for a minimum of 2×10^{10} normal cells per day, a figure which can usually be achieved using filtration leukapheresis or a continuous-flow cell separator with steroids and a sedimenting agent (Russell and Powles, 1976).

Choice of Collection Procedure

Any decision to provide a granulocyte transfusion service must depend not only on the likely demand but also on the finances available and the other purposes for which the equipment would be used. Economic arguments for such a service can be reinforced by considering the potential saving of money spent, for example, on antibiotics and additional hospitalization.

The table is intended to be a very rough guide to the relative merits of the various available techniques. If granulocyte transfusion is to be the only function performed by the equipment then economically it is impossible to consider any method other than filtration even if the cells so obtained are damaged to some extent. This is because adequate yields are obtainable using a machine which can be constructed by any competent workshop bringing this method within the reach of any large general hospital. Alternatively, a relatively cheap portable device comprising a rotary pump linked to a bubble detector and warning system is commercially available¹. A unit such as ours, which already possesses centrifugal cell separators, may still prefer to use filters because of their convenience and because of reservations concerning the use of sedimenting agents in normal donors, although there is no evidence at present that these are harmful.

The cost of buying and operating a cell centrifuge is high so these machines should be bought only if they will be used for other functions in addition to

granulocyte collection. The Haemonetics Separator was originally designed for platelet collection, a function it performs extremely well (Tullis *et al*, 1971; Szymanski *et al*, 1973). It is a valuable instrument to have if provision of a platelet support service is the primary consideration. If sedimenting agents are used, this centrifuge can provide yields of granulocytes which are at least adequate for children (Scarffe, 1975). The pumps on this machine can also be used for the filtration method. The continuous-flow cell separators, although somewhat more versatile, are considerably more expensive and should only be purchased if required to perform other functions, such as removal of acute leukaemia cells for immunotherapy and plasma exchange in patients with paraproteinaemias (Powles *et al*, 1971; Powles *et al*, 1974).

Conclusion

We feel that there is now enough evidence to suggest that granulocyte transfusion is a valuable procedure in certain clinical situations. However, there is still a need for controlled trials to define more precisely those situations in which this form of treatment can be used to the greatest advantage. For example, it would be useful to know if prophylactic granulocyte transfusions are needed in addition to other preventive measures during marrow transplantation, or if early treatment of certain specific infections, such as perianal cellulitis, could make enough difference to consider using them as a routine in such conditions.

The aim of any unit involved in a cytotoxic chemotherapy programme for haematological malignancy should be to produce a situation in which very few patients with controllable malignant disease die of infection. The addition of granulocyte transfusions to a good programme of supportive care may help to achieve this aim.

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¹Fenwal Ltd, Travenol Laboratories, Thetford, Norfolk IP24 3SE.

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