Immunological factors in nutritional assessment

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Multiple studies have demonstrated that malnutrition is one of the major causes of increased morbidity and mortality among hospitalized patients. A variety of anthropometric, biochemical and immunological parameters has been used as indicators of protein–energy malnutrition. However, the relative value of each of these measurements has not been clearly defined. This is especially true for the immunological parameters, where abnormalities are often observed in clinical and subclinical malnutrition.

The purpose of this paper is to review and discuss some of the most significant results relating to (1) the effects of malnutrition on the immune processes and (2) the effectiveness of immunological testing and assessment of nutritional status to identify high-risk patients.

Effects of malnutrition on the lymphoid organs

In malnutrition the primary lymphatic structures of the immune system, notably the thymus, as well as such secondary structures as the spleen and lymph nodes, are significantly altered in size, weight, architecture and cellular components.

The thymic-dependent areas are especially affected. Germinal centres are usually reduced in number, but occasionally are normal or even increased. There is poor documentation of the structural alterations in the gut-associated lymphoid tissues in malnutrition. The tonsils and adenoids are often small to the point of being vestigial.

In our laboratories, lymphoid tissues of six malnourished dogs were histologically evaluated and compared to the same lymphoid structures of dogs nutritionally repleted by means of total parenteral nutrition (TPN). The results showed that thymus, spleen, lymph nodes and Peyer's patches were markedly atrophic and severely cell depleted in malnourished dogs, and provided evidence that subsequent TPN therapy stimulates and supports lymphoid cell proliferation, leading to cell repopulation of previously depleted lymphoid structures (Table 1) (Dominioni *et al.* 1976).

Effects of malnutrition on phagocytic activity

As early as 1946, it was shown that protein–energy malnutrition in rats inhibited the phagocytosis and killing of bacteria by peritoneal cells (Guggenheim & Buechler, 1946). Many recent studies have investigated the production, mobilization and other aspects of phagocytic function in malnutrition.

The total leucocyte count in nutritional deficiency is usually increased or normal. Acute sepsis in malnourished patients can reduce the number of total white cells,

Table 1. *Histological evaluation of lymphoid tissues from malnourished and total parenteral nutrition repleted dogs*

(Figures indicate mean percentage lymphocyte depletion ± standard deviation)

Lymphoid structures	Malnourished dogs (%)		TPN-repleted dogs (%)		Significance of difference (Student's t test)
	Mean	SD	Mean	SD	
Thymus	50	22	41	16	NS
Peyer's patches	40	21	27	8	NS
T-dependent areas of lymph nodes	59	17	23	5	$P<0.01$
B-dependent areas of lymph nodes	45	13	47	12	NS
T-dependent areas of spleen	61	12	51	13	NS
B-dependent areas of spleen	26	4	35	11	NS

NS, not significant

including PMN leucocytes. Selvaray & Bhat (1972) have shown abnormalities of the phagocytic capacity, an intrinsic defect in the glycolytic pathway and a defect in intracellular killing of bacteria by neutrophils from patients with kwashiorkor. Recently, Wunder *et al.* (1978) examined the effects of varying protein levels in defined animal diets and showed that protein deficiency alone is primarily responsible for the depression of phagocyte function. However, the microbicidal defect in malnutrition is reversed completely when the individual's nutritional status is restored to normal (Seth & Chandra, 1972; Chandra, 1977).

Effects of malnutrition on lymphocyte functions

T lymphocytes. Studies by Chandra (1974*a*) and Ferguson *et al.* (1974) showed that the percentage of T cells in the peripheral blood is reduced in malnutrition. This reduction parallels the severity of weight loss, impaired cutaneous delayed hypersensitivity response to dinitrochlorobenzene (DNCB), and decreased DNA synthesis by lymphocytes stimulated with phytohaemoagglutinin (PHA). The abnormalities were quickly and completely reversed with nutritional improvement, thereby ruling out any primary defect of the thymus. Lymphocyte blastogenic response to PHA has been studied during malnutrition and found to be reduced (Chandra, 1975*a*; Dionigi *et al.* 1975). It is important to note that lymphocyte proliferation is reduced even in marginal undernutrition (Chandra, 1979*a*).

Results from nutritional studies performed on rats (Floyd *et al.* 1979) suggest that suppression of lymphocyte PHA blastogenesis during protein depletion involves a serum factor for the initial 3–4 weeks. However, after 5–6 weeks of protein depletion an intrinsic lymphocyte defect develops that can be corrected by nutritionally repleting the rats with a regular diet for 3 weeks.

Terminal deoxynucleotidyl transferase (TdT) is present in large amounts in the early stages of T lymphocyte differentiation. The enzyme content progressively decreases with cell maturation and fully differentiated T cells with surface

receptors for sheep red blood cells (SRBC) contain very low levels of TdT. In malnutrition, thymic hormone activity is reduced (Chandra, 1975*b*) with resultant maturational defects. There is an increase in the proportion of 'null' cells (Chandra, 1977) the majority of which may be immature T cells. TdT is increased in protein–energy undernutrition and there is a direct correlation between the number of null cells and leucocyte TdT levels (Chandra, 1979*c*). Thus TdT concentration can be used as an indirect measure of impaired T cell differentiation and nutritional deficiency.

Delayed hypersensitivity. The response to antigenic stimulation during malnutrition is variable depending somewhat on the antigen (Passwell *et al.* 1974). Primary antibody responses are usually depressed to most of the antigens, especially those requiring T and B cell co-operation; secondary responses are usually less affected. Recent studies employing a battery of antigens have confirmed the frequent occurrence of depression of delayed hypersensitivity response during undernutrition (Chandra, 1974*b*). Cutaneous delayed hypersensitivity was reduced both in terms of decreased diameter of induration and percentage of non-responders. Nutrition-related cutaneous anergy is not confined to the severe deficiency syndromes observed in developing countries. Meakins and co-workers (1977) performed delayed hypersensitivity skin testing on more than 500 surgical patients and demonstrated that anergy and relative anergy were associated with malnutrition, sepsis, shock, and trauma; in their studies, the maintenance of body cell mass by the use of TPN was associated with reversal of the anergic state and an improved prognosis.

In our centre, the delayed hypersensitivity response (DHR) of pre-operative cancer patients has been studied to evaluate possible relationships between DHR, malnutrition and post-operative infections (Dionigi *et al.* 1979*a,b*). The skin of 177 patients with the pre-operative diagnosis of cancer was tested before surgery with recall antigens (purified protein derivative, Trichophyton, *Candida* and streptokinase–streptodornase) and with epicutaneous sensitization and challenge with primary antigen (DNCB). The over-all responses were graded as normoergic, hypoergic or anergic. To identify possible causes of anergy and infection, the other parameters considered were pre-operative serum albumin level, tumor stage, duration of anaesthesia and operative contamination. 41% of the patients were recorded as normoergic, 50% as hypoergic and 9% as anergic. One or more infectious episodes occurred in 36% of the normoergic patients, in 49% of the hypoergic patients, and in 86% of the anergic patients. The incidence of infection in anergic patients was significantly higher than that in normoergic or hypoergic individuals. Albumin concentration was significantly lower in anergic than in normoergic patients. No correlation was found between the response to the skin tests and the stage of tumor. The incidence of respiratory infection was significantly higher when the duration of anaesthesia was longer than 2 h. In normoergic patients, the degree of operative contamination was associated with a significantly higher incidence of infection, whereas in anergic patients the incidence of infection was not different between clean and contaminated

procedures. It is clear, therefore that cancer patients who are pre-operatively anergic to skin tests present a higher risk of developing post-operative infection, regardless of the degree of contamination. This study also indicated that nutritional deficiency may influence some aspects of the immunological process, which may contribute to the development of infection or its progression.

B lymphocytes, immunoglobulins and complement. The percentage of B lymphocytes in the peripheral blood in malnutrition is normal or increased. An increase is often seen in those children who have an obvious infection associated with nutritional deficiency (Bang *et al.* 1975). Hypogammaglobulinemia is usually associated with severe uncomplicated undernutrition, but children with malnutrition may have elevated levels of immunoglobulins as a consequence of repeated infections. In general, IgM levels are higher than age-matched normal controls. Levels of IgA are variable but often elevated with concomitant infection, and IgE is frequently markedly elevated as a result of parasitic infections. Chandra (1975*b*) postulated that in undernourished individuals the recurrent protracted infections of the gastrointestinal and respiratory tracts occur mainly as a result of impaired mucosal immunity in undernutrition and that such infections, as well as constant immunological stimulation by the absorbed macromolecules, contribute to policlonal hyperglobulinemia. Results obtained in our laboratory showed that the average IgG levels slowly and constantly decreased during the malnutrition period, reaching a significant low concentration after 5 weeks of undernutrition. Malnutrition caused a statistically non-significant reduction in IgM concentration, whereas levels of the third component of complement showed a marked fall.

A defect in the complement system is associated with an increased susceptibility to bacterial infection (Mata *et al.* 1972). Because children with protein–energy malnutrition have an increased incidence of bacterial infections, it may be expected that their complement system is impaired. Kenny *et al.* (1965) observed a significant decrease in the serum of properdin in protein-depleted rats. Moreover, Suskind *et al.* (1976) have shown that patients with kwashiorkor have depressed haemolytic complement levels, as well as depressed levels of all complement components except C_4 but including factor B. According to Chandra (1975*c*), low levels of complement activity in nutritional deficiency may reflect a general reduction in protein synthesis or a mobilization of the limited synthetic ability for production of more urgently required antibodies directed against the invading pathogen. This observation has been recently confirmed by Wunder *et al.* (1978) who examined the effects of varying protein levels in defined animal diets and found that immunoglobulin synthesis may take priority over production of certain complement components. In the study by Chandra (1975*c*) it was found that levels of the third component of complement C_3 show a marked fall to 61% of baseline values after 4 weeks of malnutrition. In a clinical study performed in patients with oesophageal carcinoma, suffering from protein–energy malnutrition and shown to be in negative nitrogen balance, Haffejee & Angorn (1979) did not show significant differences in the levels of C_3, C_4 and C_3PA between these patients and controls. This seems to be contrary to our findings from experimental work performed on

dogs. The extent of the neoplastic process may influence the level of complement activity and this may explain the conflicting results. The great variability of the complement components concentration in studies performed in patients could also be due to different degrees of reduced synthesis as well as increased breakdown.

Effects of malnutrition on acute-phase proteins

One of the most consistent features of the body's response to tissue damage is represented by a transient but substantial alteration in the concentration of several plasma proteins. Post-traumatic metabolic alterations do not acutely affect all proteins (Alper, 1974), however, some of them (mainly glycoproteins) present a significant increase in the plasma and therefore, they have been defined as acute-phase proteins (APP). About thirty APP have been identified either in man or in experimental animals. APP are heterogeneous from the point of view of their physico-chemical and biological properties (molecular weight, site and rate of synthesis, half-life, electrophoretic migration), and they present many different and sometimes opposite biological effects. Some of them, like C-reactive protein, have been known for decades and have been used to monitor the state of activity of infectious and inflammatory disease (Hurlimann *et al.* 1966). Other APP have more recently come to the attention of clinicians because of their potential use in the staging and monitoring of malignancy (Ward *et al.* 1977) and for the early diagnosis of post-operative complications (Pantano *et al.* 1980). Elegant experiments by Miller & John (1970) have illustrated that liver synthesis of plasma proteins is finely regulated by nutritional and hormonal factors. These authors showed that in the isolated perfused rat liver the combination of amino acids, glucose and insulin is necessary in order to obtain positive nitrogen balance; moreover, the optimal conditions for the maximum synthesis of APP (fibrinogen, α-1-acid glycoprotein and haptoglobin) were found when the liver was from a fed donor animal and when a nutritionally complete intravenous solution was infused. The synthesis of albumin is also known to be depressed during malnutrition and under catabolic conditions (Rothschild *et al.* 1972a,b), however, it has been shown experimentally that after a 6 d fasting period albumin synthesis is proportionately more depressed than that of APP (Miller & John, 1970). It may, therefore, be speculated that in catabolic states the synthesis of APP has priority and this might partly account for some of the features of the plasma protein profile observed during the post-injury acute phase response. It is well known that malnutrition negatively affects protein synthesis and it has been shown in man that nutrition is probably the single most important factor regulating albumin synthesis (Rothschild *et al.* 1972a). A negative effect of malnutrition on the APP response has been observed in experimental animals by Neuhaus *et al.* (1963) and by Mouray (1966), however, there are no controlled studies in man on the effect of malnutrition on the acute phase response of plasma proteins.

Fibronectin. Plasma fibronectin, an opsonic glycoprotein of 440 000 dalton molecular weight, modulates reticulo–endothelial (RE) clearance of non-bacterial test particles, fibrin monomer and some bacterial species (Saba, 1970; Saba &

Jaffe, 1980). Fibronectin depletion correlates with RE phagocytic clearance depression, and restoration of circulating levels is associated with restoration of RE function. Opsonic fibronectin deficiency exists in septic injured patients with host defence failure. It has been shown that during starvation serum fibronectin falls by 25–30% and after refeeding increases to normal levels. For this reason, this protein also has been proposed as a sensitive index of nutritional depletion and repletion (Howard *et al.* 1981). These findings have been confirmed by others (Scott *et al.* 1981) who observed that fibronectin levels significantly dropped in obese patients who underwent a 21 d fast. At the end of the fast fibronectin levels dropped by an average of 40%.

Vitamin deficiencies and immune function

Retinol deficiency is consistently synergistic with infectious disease, even if this deficiency has only mild effects on specific antibody response. A defect in phagocytic function and properdin levels could be responsible for the association of infection with this deficiency. Retinol may be important in maintaining adequate serum concentrations of non-specific opsonins, since its administration elevates properdin levels in serum. Pyridoxine (B6) deficiency causes a significant depression in antibody formation, impairs nucleic acid synthesis, and causes a depression in delayed-type hypersensitivity reactions. The impairment of nucleic acid synthesis shown by Axelrod (1971) has a deleterious effect on cell multiplication and protein biosynthesis.

Riboflavin deficiency is also associated with a moderate depression of antibody formation and is apt to aggravate infectious disease.

Thiamine deficiency causes an increase in susceptibility to bacterial infections, but is often antagonistic to infections of viral etiologies. Pantothenic acid deficiency also causes a marked reduction in antibody formation and a decreased population of antibody-forming cells, as determined by the plaque assay. There is no evidence for a defect in antigen processing or in protein synthesis *per se*; consequently, Axelrod (1971) suggested that the antibody-synthesizing cells in pantothenante-deficient animals are somehow defective in their secretory mechanisms. A deficiency in vitamin C results in a striking tendency for infection, primarily by an influence on reparative processes, but several studies suggest that such deficiencies depress phagocytosis by neuthrophils (Nungester & Ames, 1948). Moreover, the number of macrophages in the peritoneum of vitamin C-deficient guinea-pigs is reduced and they appear smaller. In addition, the random migration of these macrophages is considerably depressed (Ganguly *et al.* 1976). Whether large amounts of vitamin C are beneficial in preventing infection remains a topic of considerable controversy. Deficiencies of other vitamins either have not been studied sufficiently for their influence on the immune response or have a variable effect.

Trace elements deficiencies and immune functions

The relationship between mineral deficiencies and host resistance is still poorly studied. Zinc-deficient animals and children have atrophy of the thymic lymphatic

system, depressed cell-mediated immunity and increased susceptibility to infection. Immunological testing in zinc-deficient mice has shown deficiencies of both cellular and humoral activity (Fraker *et al.* 1977; Fernandes *et al.* 1979). In zinc-deficient animals there is a progressive inability to mount plaque forming cell responses in the SRBC immunization assay. Primary antibody response is substantially reduced, while the secondary response, requiring helper T lymphocytes, is essentially eliminated (Good *et al.* 1979).

Zinc is essential for the appropriate function of more than seventy enzymes and initiates the action of at least thirty-five of these, including DNA polymerase, RNA polymerase and thymidine kinase (Kirchgessner *et al.* 1976). These enzymes, that participate in DNA and RNA synthesis, play a major role in both cellular and humoral immunological responses, which depend greatly on rapid proliferation of immunocytes and protein synthesis by these cellular elements. Lennard *et al.* (1974) have shown in humans that there is a correlation between the zinc content of phagocytes and their bactericidal activity. More recently, Golden & Golden (1978) skin-tested ten children with *Candida* antigen on both arms to see if depression of cell-mediated immunity in malnourished children was also associated with zinc deficiency. One test site was covered with local zinc sulphate and the other with a placebo ointment. There was a highly significant increase in the typical delayed hypersensitivity reaction at the site covered with zinc, showing that zinc deficiency is a cause of the immune incompetence seen in malnutrition.

Investigations examining iron and iron-binding proteins show that this trace metal plays a role in the control of lymphocyte circulation (De Sousa, 1978) and distribution (De Sousa *et al.* 1978). It has been also shown that iron causes an impairment in E rosette formation by T lymphocytes, and that iron bound to lactoferrin confers an ability to regulate colony forming cell differentiation in vitro (De Sousa *et al.* 1978). Iron deficiency may depress bactericidal activity of leucocytes in experimental animals and man. This abnormality is rapidly corrected by administration of iron, indicating that iron deficiency is another factor in the production or potentiation of immune-deficient states. The competition between host and micro-organisms may play a role in the non-specific host resistence to infection, and the virulence of the micro-organisms is partially determined by its success in binding such metals as iron, copper and zinc. An important defence mechanism of the host against microbial multiplication is the iron-binding proteins, such as lactoferrin and transferrin, both of which may inhibit microbial growth by reducing the availability of iron. In breast milk, for example, lactoferrin and transferrin are strongly bactericidal and bacteriostatic to *E. coli* if unsaturated with iron. Serum transferrin is diminished in protein malnutrition, presumably due to impaired synthesis. If available, transferrin becomes highly saturated with iron; relatively high levels of free iron are available for promotion of bacterial growth and may result in overwhelming infection and death (Neumann *et al.* 1975). There remains much to be discovered about the role of iron-binding proteins in the resistance to infection, but there is evidence to suggest that they could be of crucial importance.

Overnutrition

In affluent societies, obesity is the commonest type of malnutrition. Earlier studies reported an increased incidence of respiratory infections and post-operative sepsis in the obese, often resulting in more prolonged hospitalization. Infection is a frequent primary or contributing cause of death in the obese. The mechanisms underlying increased infection complications and mortality in obesity are largely unknown. A recent survey revealed subtle deficits in cell-mediated immunity and polymorphonuclear leucocyte function which were correlated with associated micronutrient deficiencies (Chandra, 1979*b*). Hyperlipidaemia is a frequent complication of obesity and high lipid levels may influence immune responses. Serum containing high levels of cholesterol and low-density lipoproteins can inhibit in vitro lymphocyte transformation responses to mitogens and phagocytosis by polymorphonuclear leucocytes.

Immunological abnormalities have been demonstrated in obesity also (Chandra, 1981). A proportion of obese adolescents and adults showed a variable impairment of cell-mediated immune responses in vivo and in vitro and reduction of intracellular bacterial killing by polymorphonuclear leucocytes. In genetically obese mice, the number of mononuclear cells and thy1,2–positive lymphocytes in the thymus and the spleen was less, compared with that in lean controls. The plaque-forming antibody response was reduced and the cytotoxic response of spleen cells of obese animals immunized in vivo was markedly lower than that of lean controls, whereas the same response after in vitro sensitization was normal.

Immunological testing and evaluation of nutritional status in cancer patients

The purpose of the immunological studies in cancer patients is to achieve a better understanding of the pathophysiology and natural history of neoplasms; furthermore, careful analysis of immunological information may offer guidelines for selection and modulation of appropriate anticancer therapy. Moreover, cancer treatment has to be associated with nutritional support, which requires markers of the need for the treatment and the effectiveness of it. Concomitant determinations of a few selected immunological and nutritional indicators is also helpful to identify high-risk cancer patients, who could develop major complications during treatment, i.e. post-operative infections.

Immunological parameters. Literally hundreds of tests can be used to survey the immunological status of cancer patients. These tests are tedious and time consuming and require a laboratory dedicated to excellence. However, there is no reason why they cannot be performed routinely in most major hospitals dealing with this patient population, since immunological surveillance can minimize the risk of surgical procedures and their complications. The immunological tests which are usually included in nutritional assessment, since they are supposed to have correlations with the nutritional status of the patient, will now be discussed as will the clinical relevance of some of these tests in identifying the high-risk cancer patients.

An emerging application of the measurement of DHR is its use as an indicator of nutritional status (Daly *et al.* 1979; Dionigi, Dionigi *et al.* 1980). The correlation between the impairment of DHR and a definitely worse prognosis has been observed repeatedly in cancer patients and also in patients with non-neoplastic critical illness (Meakins *et al.* 1977; Harvey *et al.* 1979). In spite of the relevant prognostic values of depressed DHR, the etiology of anergy to skin tests in cancer patients is unclear. There is little doubt that the cause of depressed DHR is multifactorial, since at least five possible causative conditions are presently known: (1) pre-existing primary or acquired immunodeficiency, (2) the presence of cancer *per se*, (3) malnutrition (either primitive or cancer-induced cachexia), (4) advanced age, (5) previous immunosuppressive therapy.

The evidence supporting the determination of DHR for the purpose of nutritional assessment is inadequate. For this reason in our Institution a study was carried out to evaluate the separate role of malnutrition, advanced age and stage of tumor growth as causes of depression of DHR. The study was performed on 111 surgical patients with solid tumors and on fifty-six non-neoplastic control patients, matched for age, sex, anatomical site of disease, degree of ill-health and nutritional status. The DHR of cancer patients was not found to be significantly different from the DHR of these carefully matched controls; in both cancer patients and controls the DHR was significantly depressed as compared to the DHR of younger hospitalized individuals with minimal disease. These observations suggested that cancer patients and matched controls shared some non-specific immuno-suppressive conditions, such as advanced age and malnutrition.

In the cancer patients the progressive growth of neoplasia was significantly correlated with, and apparently was the cause of, malnutrition, as measured by the decrease of serum albumin level.

A decrease of serum albumin level, which represents an index of established malnutrition, was shown to correlate with depressed DHR both in cancer patients and in controls. These results indicate that the impairment of DHR in cancer patients is a consequence of tumor-induced malnutrition rather than a pre-existing immuno-deficiency; this concept is further supported by the finding that well-nourished cancer patients did not present a DHR different from that of well-nourished non-neoplastic controls matched for age, sex and site of the disease. Only in malnourished cancer patients did the stage of tumor correlate with the DHR, suggesting that tumor growth interferes with the development of DHR only when it causes malnutrition. A significant correlation was found between the age of the patients and the decrease of their DHR, confirming previous reports which showed that the process of ageing in otherwise healthy subjects is accompanied by a progressive impairment of cell-mediated immunity (Gross, 1965; Teasdale *et al.* 1979). In another study (Dionigi *et al.* 1979*a*) DHR has been evaluated and we have been able to show that in cancer patients undergoing surgery, septic complications occur in 37% of the normoergic, in 46% of the hypoergic and in 83% of the anergic patients, the incidence of infections in anergic patients being significantly higher than in those who were normoergic or hypoergic. The

impairment of cellular immunity determined by DHR correlated with a high incidence of infective complications in the post-operative period. This observation is in agreement with the results reported by Mullen and co-workers (1979), who found that the most common complication in those patients who were malnourished was sepsis.

The results obtained in the study of DHR by means of skin testing are still conflicting and the real immunological meaning of this parameter is unclear. Moreover, the results presented by different study groups are not always comparable because of several methodological discrepancies. In fact the definitions of normoergic, hypoergic or anergic states is often different since some authors establish the efficiency of the immunocompetence administering only recall antigens which allow the study of the secondary immune response, whereas others evaluate primary and secondary immune responses by means of primary (DNCB) and recall antigens. In our experience the over-all evaluation of skin test response to DNCB and recall antigens is more complete and discriminating, in fact patients who could be hypoergic or anergic if evaluated only with recall antigens are normoergic if tested with DNCB. Evaluation of DHR by means of recall antigens presents also other problems of interpretation, since the skin test response could depend on the epidemiology of the used antigen. In fact the use of the same type of antigens is probably wrong, since the incidence of many infectious diseases varies in different countries. For this reason we recently decided to evaluate the role of each single antigen in the definition of the type of response in order to possibly eliminate those antigens which do not significantly contribute in the evaluation of the immunological response. In patients with lung cancer 199 determinations have been performed; ninety responses were recorded as normoergic and 109 as hypoergic. In the normoergic group DNCB was always essential for the diagnosis of a normoergic response, protein purified derivative (PPD) was essential in 22% of the cases, varidase in 7%, whereas *Candida* and Tricophyton were practically not essential since the former was essential in only 1% of the cases and the latter not at all. Similar results have been obtained with the hypoergic response, where DNCB was essential in all the cases (100%), PPD in 22%, varidase and Tricophyton in 0·9% and *Candida* by itself never contributed to a hypoergic response. These results show that in patients with lung cancer the normoergic and hypoergic responses could be defined using only DNCB, PPD and varidase; Tricophyton and *Candida* could be abandoned. In our opinion the selection of a battery of recall antigens should be done in each distinct geographical area on a large number of normal volunteers.

In a recent study performed in our laboratories, sequential determinations of circulating lymphocytes, T lymphocytes, in vitro lymphocyte blastogenesis, C_4 and factor B have been carried out in fifty-six patients of both sexes undergoing surgery for the removal of primary localized malignant neoplasms of the breast, stomach, or colon–rectum or melanoma. All clinically relevant infectious episodes occurring in each patient until post-operative day 14 were recorded carefully, and sepsis was diagnosed only when positive cultures supported the clinical suggestion

of infection. Patients who underwent radical mastectomy had an uneventful post-operative course; among the patients who had excision of melanoma, 15% developed infections. After gastric resection, 35% presented one or more localized sepsis (respiratory, urinary or thrombophlebitic). Among the patients who underwent resection of the colon–rectum, 73% developed one or more infections (of the wound, urinary or respiratory tracts or intra-abdominal abscess). In all four patient groups the majority of infections (74%) were diagnosed within 7 d of surgery and could be satisfactorily treated by antibiotic therapy and appropriate post-operative supportive care. In no instance did generalized sepsis develop. 90% of infections were caused by gram-negative bacteria, the more commonly isolated being *E. coli* (36%) and *Proteus* (27%) organisms.

Pre-operative and post-operative determinations of lymphocyte and T lympho-cyte levels show, in patients who subsequently developed infections, moderately low values. Non-significant differences between septic and non-septic patients have been observed in lymphocyte blastogenic response to phytohaemoagglutinin in vitro. Nevertheless, all three parameters of cell-mediated immunity show a constant depression during the first post-operative week. A more accurate analysis of the results, which take into consideration different groups of patients according to the type of malignancy and surgical procedure, shows that the lowest levels of lymphocytes and T lymphocytes observed during septic complications occur in patients undergoing resection of the colon–rectum. Baseline levels of C_4 were significantly elevated in all patient groups, whereas levels of factor B were significantly higher only in patients with cancer of the stomach and colon–rectum; these results confirm the findings of Verhagen *et al.* (1976). After radical mastectomy and after excision of melanoma, the levels of C_4 increased promptly and significantly ($P<0.05$) and remained elevated throughout the 2 week period of the study. In contrast, after gastric resection or resection of the colon–rectum, C_4 levels did not increase until the second post-operative week. The level of factor B showed similar post-operative variations. The separate plotting of the curves of septic and non-septic patients with gastric cancer demonstrated that patients without post-operative sepsis have an increase of C_4 and factor B, whereas patients with sepsis do not. In the other groups, the majority (78%) of patients with post-operative infections had lower levels of complement than did non-septic patients.

Our findings provide further evidence that many bacterial infections in humans can result in consumption of opsonic proteins that are critical for antibacterial defence. Alexander (1974) has termed this process a 'consumptive opsoninopathy' and showed that severe bacterial infections cause a consumption of opsonic proteins that may reduce the ability of the patient's serum to opsonize bacteria, thereby further increasing susceptibility to infection (Alexander *et al.* 1976).

Anthropometric and biochemical measurements

Nutritional assessment should be an integral part of the evaluation of those hospitalized patients who are scheduled for surgical procedures. To identify the

Table 2. *Mean value of nutritional and immunologic measurements*

	Controls (n 21)		Cancer patients (n 71)		
Anthropometric measurements	Mean	SE	Mean	SE	Significance (Student's *t* test)
% Ideal body-weight	103·6	16·2	108·0	21·4	NS
% Usual body-weight	100·8	3·3	94·8	9·4	P⩽0·01
% Weight loss/month	0·2	0·8	1·6	0·8	NS
% Standard arm circumference	102·1	10·9	97·3	13·8	NS
% Standard triceps skinfold	102·3	44·2	120·5	51·0	NS
Visceral protein compartment					
Haematocrit (%)	43·5	2·5	39·0	4·8	P⩽0·001
Haemoglobin (g %)	14·6	1·1	12·8	1·8	P⩽0·001
Serum proteins (g %)	7·1	0·7	6·9	0·9	NS
Albumin (g %)	4·1	0·5	3·6	0·6	P⩽0·01
Fe (µg %)	132·6	49·8	74·7	39·7	P⩽0·001
Transferrin (mg %)	271·4	44·9	262·0	71·4	NS
Ceruloplasmin (mg %)	38·0	4·2	45·8	9·7	P⩽0·001
Retinol binding protein (mg %)	5·5	0·9	4·6	1·9	P⩽0·05
Lean body mass					
Serum creatinine (mg %)	0·8	0·1	0·8	0·2	NS
Urine creatinine (g/die)	119·1	465	978·0	648	NS
Creatinine/height index (%)	76·5	30·3	73·7	44·7	NS
Arm muscle circumference (%)	101·7	12·6	93·2	10·7	P⩽0·01
Immunological status					
Lymphocytes (/mm³)	2797	731	2356	861	NS
White blood cells (/mm³)	6714	1181	6661	2096	NS
C3c (mg %)	94·4	17·3	106·3	26·3	P⩽0·05
Skin tests (% normoergic)	100		60·3		P⩽0·001

NS, not significant

malnourished patients, Blackburn & Bistrian (1976) were pioneers in developing and defining detection methods. A variety of anthropometric and biochemical measurements have been found to be of considerable value in recognizing malnutrition in hospitals. Nevertheless, the clinical relevance of each of these proposed parameters are not yet firmly established, and only recent results show that some specific markers could be predictors of septic complications (Meakins *et al.* 1977; Dionigi *et al.* 1979*b*). The parameters used in our Institution for nutritional assessment are shown in Table 2.

In a recent study (Dionigi, Dionigi *et al.* 1980) the complete pre-operative nutritional assessment was performed to evaluate nutritional assessment modifications in twenty-one control patients with benign minor surgical diseases and in seventy-one surgical cancer patients. Determination of the relative value of these twenty-one indicators known to be affected by protein–energy malnutrition in relation to the post-operative septic complications in cancer patients was also performed (Table 2). Ten of these parameters seem to be useful in the assessment of the impaired nutritional status of cancer patients. The significant variation of the percentage of usual body-weight observed in this patient population shows that changes in body-weight still remain a useful indicator of the modifications in over-

all nutritional status. This result confirms previously reported findings of Bistrian and co-workers (1974) that weight loss is a reliable index of impaired nutritional status especially when lean body mass is reduced.

Skeletal muscle stores, as measured by mid-arm muscle circumference, were lower in cancer patients when compared with controls. The muscle circumference of the mid-arm provides a good index of the whole body skeletal muscle; nevertheless, in order to reduce observer variability it requires standard protocols on a repeated daily basis. Severe depletion of skeletal muscle mass, defined as less than 60% of normal, is not so common in cancer patients; in fact, in our study, none of the patients has a severe depletion. In the study by Bistrian *et al.* (1974) 20% of the cancer patients had an arm muscle circumference of below 60% of normal; this difference could be due to the stage of malignancies and their localization. Plasma albumin was significantly decreased in cancer patients indicating that this measurement is a reliable and sensitive discriminant of deteriorating nutrition (Whitehead *et al.* 1973) even if we did not observe any relationship with infective complications. One of the limitations of using albumin as a nutritional indicator is that its measurement is inadequate for detection of early deficits. In fact, there is a considerable capacity of adaptation in albumin metabolism as serum albumin concentration falls (James & Hay, 1968). Recent results suggest that proteins with a short half-life, such as transferrin and retinol-binding protein (RBP) (Ingenbleek *et al.* 1975) could reflect malnutrition more rapidly. The normal transferrin mean concentration observed in our study could be explained by the particular behaviour of transferrin; in protein deficiency, serum transferrin concentration falls, whereas in iron deficiency it rises (Baum, 1973). In fact in our study we noted in the cancer patient group a normal transferrin mean concentration and a significant iron deficiency.

In a recent study Young & Hill (1978) included serum levels of ceruloplasmin, an α_2-globulin synthesized in the liver, in the nutritional assessment of fifty-four randomly selected surgical patients; no significant variation was found. Contrary to these results, we observed a highly significant increase in serum ceruloplasmin levels in cancer patients. The increase in ceruloplasmin confirms the observations obtained by Goulian & Fahey (1961) and Scanni *et al.* (1977) on patients with gastrointestinal cancer and lymphoma.

RBP has a very fast turnover rate and shows a high sensitivity to alteration of nutritional status. Our study showed that RBP is lower in cancer patients than in controls. Its short half-life, of about 10 h (Peterson, 1971), suggests that this index could be the most responsive to dietary manipulations. Pre-operative determinations of haemoglobin show a significant low concentration in cancer patients; this is in contrast with other findings (Young & Hill, 1978) in which normal mean values were found for haemoglobin in individuals before surgery. The anaemia observed in this cancer population could depend on the ratio of males to females, which is lower than in the control group, but it could be partially attributed to some degree of blood losses and/or impaired erythropoiesis which occurs in neoplastic diseases. The present study confirms that in cancer patients

skin reactivity to antigens is reduced (Copeland *et al.* 1976). A significant decrease in the pre-operative value of ceruloplasmin was noted between patients who developed post-operative infections and patients who did not. The mechanism which determines these variations is not completely known. Ceruloplasmin is one of the acute-phase proteins and its elevation in those patients who will develop sepsis in the post-operative period could be due to infectious episodes at a subclinical level.

The use of cluster analysis in nutritional evaluations

In an attempt to deal with the problem of arriving at data-dependent physiological groupings, Siegel *et al.* (1971) applied cluster analysis techniques to studies of physiological data from critically ill patients.

Recently, a similar study has been performed in our Institution to evaluate the nutritional status of surgical patients by means of cluster analysis in order to identify different nutritional patterns, which could condition the prognosis of the patients. The study was performed at admission and before any type of treatment on twenty-two patients with minor benign surgical diseases, without any predictable influence on the nutritional status, and on forty-nine patients with cancer (twenty-three of the gastrointestinal tract and twenty-six of other organs). The following were determined: haemoglobin, transferrin, iron, albumin, ceruloplasmin, RBP, lymphocytes, delayed hypersensitivity response to primary and recall antigens (Dionigi, Dominioni *et al.* 1980), complement component C3c, percentage usual body-weight and percentage standard skinfold triceps. Each parameter was normalized and the normalizing factors were the normal values reported in the literature taking into consideration, when necessary, the sex of the patient. The sets of data were then clustered by means of a hierarchical and ascending algorithm (Jambu, 1978). This algorithm starts from as many groups as patients and successively aggregates to arrive at one group. The computerized analysis of the data suggested four clusters.

In cluster 1 all values, except RBP and ceruloplasmin which were higher and standard triceps skinfold which was slightly lower, were in the normal ranges. Each of the other three clusters was characterized by peculiar abnormalities of certain parameters so that none of them was found to be normal in all the four clusters. Cluster 2 was characterized by an important decrease of all parameters except lymphocytes, C3c and usual body-weight, which were in the normal range and ceruloplasmin which increased notably. Particularly important was the reduction in the value for iron that reached 40% of normal. Cluster 3 was characterized by a slight and uniform decrease of all parameters except skin tests that were normal and RBP and ceruloplasmin values which were higher than normal but similar to those found in cluster 1. Major changes were found in cluster 4. Haemoglobin, skin tests, standard triceps skinfold and, particularly, iron were decreased; usual body-weight and albumin were in the normal ranges; the other parameters were all higher than normal, particularly RBP and ceruloplasmin. Table 3 shows the incidence of the variables in the clusters. Cluster 1 was

Table 3. *Incidence of clinical variables in the cluster*

	Cluster				Significance of difference (Student's t test)
	1	2	3	4	
Benign diseases (%)	50	6	—	—	$P<0.001$
Gastrointestinal neoplasias (%)	16	52	57	60	$P<0.05$
Other neoplasias (%)	33	41	42	40	NS
Post-operative sepsis (%)	26	47	57	60	$P<0.05$
Palliative procedure (%)	0	29	28	20	$P<0.001$
Severe surgical trauma (%)	38	82	85	100	$P<0.001$
Contamination of the operative field (%)	17	76	71	60	$P<0.001$
Mean duration of anaesthesia (min)	95	170	120	159	$P<0.05$
Mean post-operative hospital stay (d)	11	14	17	24	NS
Death, 6 months (%)	10	43	29	40	$P<0.05$

NS, not significant

characterized by the lowest incidence of malignancies of the gastrointestinal tract, post-operative infections, severe surgical trauma, contamination of the operative field, palliative surgical procedures and 6-months mortality. Moreover, the patients belonging to this group had the shortest duration of anaesthesia.

A progressive increase from cluster 2 through cluster 4 was observed for the incidence of cancer, cancer of the gastrointestinal tract, post-operative sepsis and severity of surgical trauma. Cancer originating outside the gastrointestinal tract had a similar incidence in the four clusters and the mean post-operative hospital stay showed an irregular distribution, however, all the other considered variables had higher incidences in clusters 2, 3 and 4 than cluster 1, the differences being statistically significant. Our results suggest that cluster analysis allows identification of four nutritional situations characterized by significantly different values of nutritional and immunological indicators. Cluster 1 can be considered as a reference group; in fact patients in this cluster have a low incidence of post-operative infections and the lower incidence of 6-months mortality. The other three clusters include patients with poorer prognoses as evaluated by the higher incidence of the variables considered. It is interesting to note the different distributions of gastrointestinal tract cancers and the neoplasias of other organs in the four clusters; in fact only the incidence of gastrointestinal tract cancers increases progressively and significantly in the poorer prognosis clusters, suggesting that this type of neoplasia is more frequently associated with nutritional abnormalities.

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