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A.O. Ajayi
Department of Microbiology,
Obafemi Awolowo University,
Ile-Ife, Nigeria

The Influence of Age-Sex on the Pattern of Immunodepression Following Surgery

A.O. Ajayi, ¹O. Adejuyigbe and O.O. Shonukan

Present study was conducted to evaluate patients for the influence of age-sex on the pattern of modulation of selected immune parameters, following surgery. Male patients, of varying ages, without any underlying immune depression and needing minor to moderate dissection were recruited randomly into the study following informed consent. They were placed in each of two groups: group 1 (GRP1) = ages 0.1 to 12 years and group 2 (GRP2) = ages 13 to 50 years. Blood samples were obtained 1) immediate preoperatively and 2) immediate postoperatively in every case and assessed for T-cell count, lymphocyte viability, B-cell count, total leukocyte count, total lymphocyte count and percentage lymphocyte transformation. T-cell counts and percent lymphocyte transformation alone were significantly different postoperatively but not preoperatively for both groups ($p=0.023$ and 0.031 , respectively). All other parameters were not. Postoperative values for T-cell counts and lymphocyte transformation were, however, lower than their corresponding preoperative levels. The results have shown that age-sex influences immune parameters such as T-cell population and function after, but not before, surgery suggesting the possible involvement of background gonadal neuroendocrinologic influences in the immune response to surgical stress.

Key words: Age-sex, immunodepression, lymphocyte transformation

INTRODUCTION

Surgery induces a depression of immune function in operated patients^[1]. This depression involves a reduction in T-cell count, depressed delayed-type hypersensitivity responses to recall antigens and a diminished lymphocyte response to mitogens *in vitro*.

Non-operative conditions such as mental stress and burns may also modulate the immune response^[2-5] by eliciting neuro-endocrine responses, which in turn depress specific aspects of the immune response^[6,7]. Also, from a psychoneuroimmunology (PNI) perspective, physiological and psychological insults of all types activate similar neuroendocrine mechanism of immunosuppression^[8].

The degree of immune depression following surgery has been related to the degree of trauma, type and duration of anaesthesia as well as to the duration of surgery^[9]. Major surgery may cause immunosuppression while minor ones may stimulate the immune response^[10]. However, surgery, whether minor or major, significantly depresses polymorphonuclear and monocyte functions and increases serum cortisol levels^[11]. Such depressions may result in postoperative sepsis, morbidity and mortality and suggest a host-related predisposition. For instance, patients who are anergic before surgery and remain so after surgery carry a higher probability of immuno-depression occasioning sepsis and death^[12]. The elderly, the severely ill patient and those with HIV infection are also at a higher risk of immuno-depression following surgery^[13]. It is also probable that other host-related factors such as the sex of the patient, by influencing the endocrine milieu, may have an effect on the immune response following trauma and surgery.

Age-sex as a combined factor has not been sufficiently investigated in human immunodepression following surgery. Some previous studies pooled data for age in a mixed sample of both sexes and vice-versa. Such heterogeneous data may not sufficiently discriminate between the contributions of age, sex or age-sex. Also possible age-related influences on the internal milieu before the institution of surgical stress were often not taken into account in the evaluation of the impact of such surgical stress on immunocompetence.

The present study investigated the pattern of immunodepression in male surgical patients with a view to determining the influence of age-sex on the immune response following surgery.

PATIENTS AND METHODS

Patients: Sixteen male patients without any underlying immunodeficiencies were recruited into the study. The age range for the patient was 0.2-50 years with a mean of

25.8 years. Types of surgery were; herniorrhaphy (35%), Lipomectomy (10%), exploratory laparotomy (10%), zygomatic bone elevation (5%), EUA and anal dilatation (5%) hydrocelectomy (5%), skin grafting (5%), left arthroscopy (5%), others (20%). The procedures were minor to moderate to minimise the degree of trauma. Mean duration of surgery was 71.3 min. Anaesthesia was general with halothane used in all cases.

Methods: Patients were assigned to one of two groups as follows: Group1: ages 0-12 years; Group 2: ages 13-50 years. About 5 mL of blood was collected by venipuncture from each patient before and after surgery. This was transferred into pre-sterilised bottles containing glass beads and promptly, defibrinated. Total Leukocyte Count (TLC) was carried out in an improved neuber haemocytometer after lyses of red cells in 1% acetic acid. White blood cell differential counts were obtained from Leishman-stained films. Total lymphocyte count was obtained numerically from both total and differential counts.

T. cell counts: T cells were separated from B-cells and other adherent mononuclear cells by passage through nylon wool columns. Columns were prepared in 5mL syringe barrels, which were packed to the 2.5 mL mark. The barrels with the wool were soaked in Hank's B.S.S. and maintained at 37°C together in a water bath prior to the introduction of the cells. 0.1 mL of defibrinated blood was loaded onto the column and incubated in the water bath for 30 min at 37°C. Slow elution with warm Hank's B.S.S. was then carried out through the nylon wool column. Eluted red cells were lysed by the addition of 0.5 mL of 1% acetic acid. Eluted T-cells were counted in a haemocytometer chamber.

Cell culture: Cell culture was undertaken in bijou bottles using the whole blood protocol of Paul *et al.*^[14] with little modification. Briefly, 0.4 mL Concanavalin A (Con A), at a final concentration of 25 µg mL⁻¹, fitter sterilised at 0.22 µ, was added to 2.3 mL aliquots of membrane sterilised culture medium (RPMI 1640, supplemented with 20 mm glutamine, 100 mg mL⁻¹ streptomycin and 100 i.u. penicillin mL⁻¹) to give 10 µg ConA per bottle. 0.3 mL of defibrinated blood was added to give a final blood dilution of 1:10 and a cell concentration of 2-3x10⁶ Lymphocytes /mL per bijou bottle. Triplicate cultures were set up including the control, which received 0.4 mL of the culture medium instead of Con A. The screw caps were tightly closed and the bottles incubated at 37°C for 72 h without CO₂.

Termination of culture and harvesting of cells: Cultures were terminated at 72 h. The cells were harvested and

washed several times in phosphate buffer pH 7.0 until whitish and were fixed in 3:1 methanol-acetic acid solution. Smears were prepared on slides, air-dried and Wright-stained.

Determination of percentage lymphocyte Transformation: Transformed (Blast) cells, easily recognised as enlarged lymphocytes with a pale blue abundant cytoplasm and an euchromatic nucleus, were counted under the light microscope's 40x objective. Hundred cells were counted at random with other non-transformed cells on the Wright-stained slides. The total number of blast cells in the count is expressed as the percentage lymphocyte transformation

Cell viability: Cell viability pre-and post-culture was evaluated using the trypan-blue dye exclusion method. After the lysis of red cells, lymphocytes were washed three times in isotonic saline and then re-suspended in Hanks' BSS. After a gentle stirring, 1.0 mL of the cell suspension was transferred to a test-tube to which two drops of 1% try pan blue dye was added. Cells that took op the dye were counted as dead. Hundred cells were counted randomly in a haemocytometer chamber and the number of living cells was recorded as a percentage

Statistical analysis: Data collected were analysed using the Statistica packages for the Life Sciences. Pre-and post-operative data for groups 1 and 2 were analysed for significant difference within and between groups. T-tests for dependent and independent variables were carried out. The test statistics for the hypothesis that the two groups were not different with respect to each of the parameters studied was then compared with the critical t-value at the 5% level of significance.

RESULTS

T-cell count and age: T-cell counts appeared higher in the age range 0.2-12 years when compared to the older patients (Fig. 1 and 2). Post-operative counts were, however, lesser (Fig. 1) with respect to their corresponding pre-operative values. Post-operative T-cell counts were significantly different between Groups 1 and 2 only ($P < 0.03$) (Fig. 3).pre-operative counts were, however, not significantly different between the groups.

Percentage lymphocyte transformation and age: This showed similar trend in distribution both pre-and post-operatively. (Fig. 5 and 6) However, percent lymphocyte transformation after surgery was significantly different between the two groups (Fig. 4) with $p=0.031$. Pre-operative values were not significantly different.

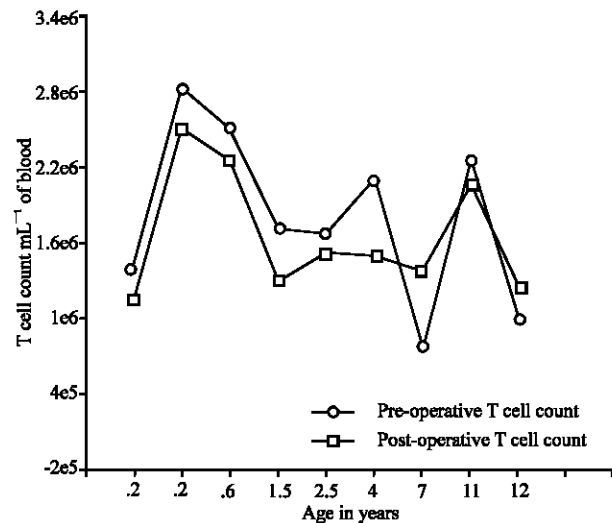


Fig. 1: Pre and post-operative T cell count for group 1

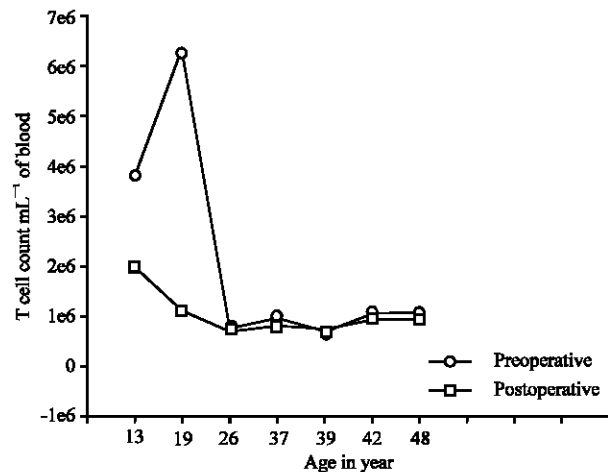


Fig. 2: Pre and post-operative T cell count for group 2

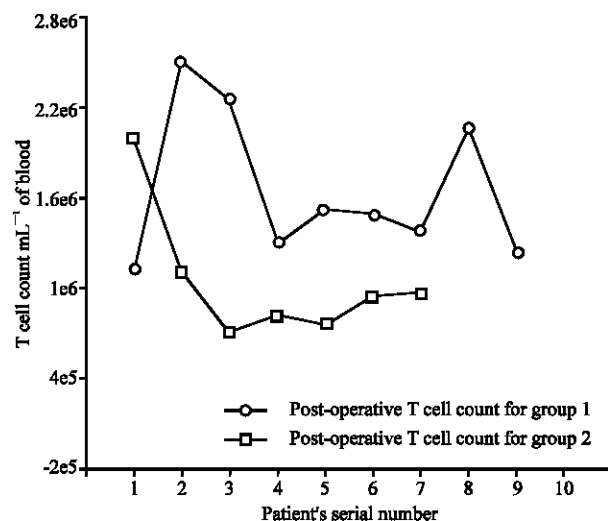


Fig. 3: Post-operative T cell count for group 1 and 2

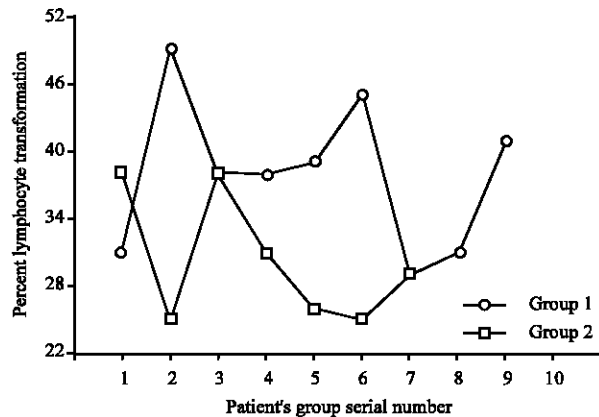


Fig. 4: Postoperative percentage lymphocyte transformation for group 1 and 2

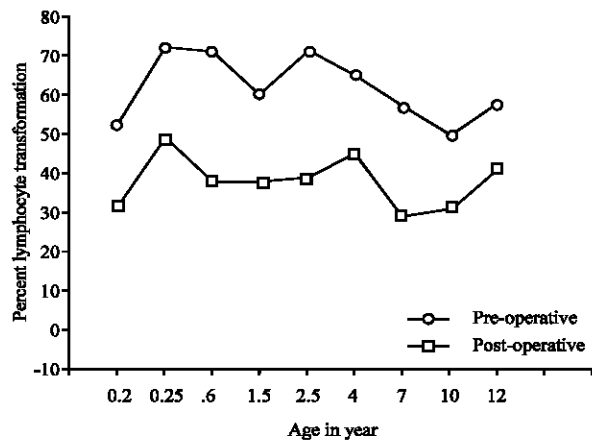


Fig. 5: Pre and post-operative percent lymphocyte transformation for group 1

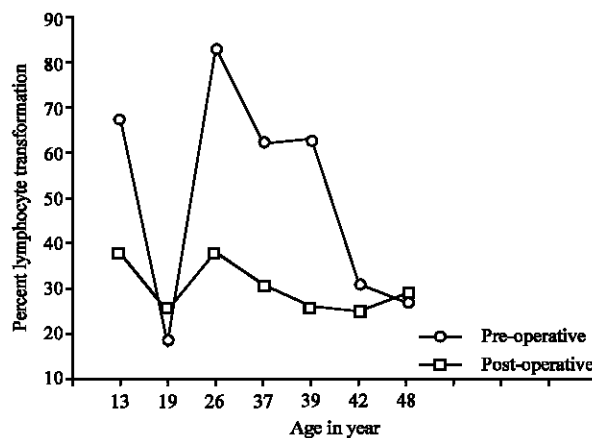


Fig. 6: Pre and post-operative percent lymphocyte transformation for group 2

Table 1: Mean values for blood parameters in Group 1

	Pre-operative	Post-operative
Total leukocytes/mL	3706111.1	4870555.6
B-cell count/mL	446127.8	732333.3
% lymphocyte viability	85.9	67.8
% lymphocyte transformation	61.7	37.9
T-cell count/mL	1619410.0	1586400.0
Total lymphocytes/mL	3277765.0	1834300.0

Table 2: Mean values for blood parameters in Group 2

	Pre-operative	Post-operative
Total leukocytes/mL	4247142.9	4320142.9
B-cell count/mL	508078.6	571207.1
% lymphocyte viability	68.9	68.4
% lymphocyte transformation	50.9	30.3
T-cell count/mL	2068200.0	1048485.7
Total lymphocytes/mL	1954142.8	1619242.9

Degree of depression of other immune parameters: The means of the data for the parameters studied before and after surgery are presented in Tables 1 and 2 for the two groups. Except for percent lymphocyte transformation and T-cell counts, the means were not significantly different between and within groups.

DISCUSSION

In spite of great advances in surgical procedures, skill and aseptic techniques, post-operative sepsis occasioning morbidity and mortality continue to pose a problem for surgeons and immunologists alike. Infections range from clinical sepsis to occult mycotic infections^[15]. The category of patients affected is varied but the elderly, the severely ill and those otherwise immuno-compromised continue to be at higher risk.

Why some patients are energetic before surgery, turn or remain anergic peri-operatively or post-operatively is not entirely clear. Pre-existing anergy, which persists post-operatively, is prognostic of post-operative sepsis and death in most cases. Altogether, these show that host-related factors affecting primary immune expression may be of some importance in surgery-induced immunodepression^[16,17].

Our results show that this indeed might be the case. Lymphocyte transformation declined proportionately in both the pre-operative and post-operative samples as age increased. This pattern of decline is independent of surgery and is more related to the age-sex of the patient. Surgery did not seem to alter the pattern but served to reset it at a somewhat lower level. This is similar to the findings of Danek *et al.*^[18] who reported two distinct patterns of polymorphonuclear and monocyte responses to injury that are independent of age, sex and severity of operation but which are thought to be associated with the degree of stress pre-operatively or with genetic factors.

Our results also showed a significant difference in post-operative lymphocyte functions as shown by the lymphocyte transformation assay. T-cell counts were also significantly different for the two groups. The reduction in the number of re-circulating T-cells observed could be due to apoptosis or alterations in chemotaxis. Alterations in functions such as phagocytosis, opsonisation and chemotaxis have been reported in burns while a reduction in CMI has been reported during surgical stress^[19].

These significant differences between the two groups, therefore, may refer to fundamental differences between the two groups in their response to surgical stress. These fundamental differences may include the relative maturity of the immune system in the older group and the influence of neuroendocrine responses. For instance, cell of the immune system such as T-cells posses receptor sites for neuroendocrine hormones and neurotransmitter molecules and vice versa. This bi-directional co-modulation of the neurohormonal and immune system and the fact that neuroendocrine responses are often age-sex related, suggest a possible role for age-sex in the immune response to surgery^[20]. In males, testosterone is the dominant gonadal hormones while oestrogen and progesterone predominate in the female. While oestrogen enhances the immune response, testosterone depresses it resulting in a sexuality-driven gradient of immune status in the individual^[21].

Post-operative immunosuppression may thus reflect a non-specific neuroendocrine stress response to surgical trauma with an impact on the balance between Th1 and Th2 lymphocyte responses^[22]. Our findings are in agreement with this. We conclude, therefore, that a pre-existing age-related down -regulation of immune function is, in the male at puberty, only further depressed by the experience of surgery and that the latter is age-sex related.

REFERENCES

1. Ignatov, I., H. Agreeva, P. Petrova and N. Stoikov, 1997. Intra-abdominal sepsis. evaluation of immunological problems and their importance for the survival and the maximal recovery. *Br. J. Surgery*, 84: Supplement 2. 89.
2. Park, S.K., J.I. Blody and H.A. Wallace, 1971. Immuno-suppressive effects of Surgery. *Lancet*, 1: 53-55.
3. Jurbert, A.V., E.T. Lee and E.M. Hersh, 1973. Effects of Surgery, Anaesthesia and intra-operative blood loss on Immunocompetence. *J. Surgical Res.*, 15: 399-403.
4. Pietsch, J.B., J.L. Meakins and L.D. Macleud, 1977. The delayed hypersensitivity response: application in clinical surgery. *Surgery*, 82: 349-355.
5. Sugar, A.M., 1989. Problems in the diagnosis of invasive candidiasis. In *Opportunistic fungal infections: Focus on Fluconazole*. London. Royal Society of Medicine Services Ltd.
6. Guenchera, G., P. Popova, F.J. Mcguidan and N. Nikolov, 1989. Experimental Stress and Immunity: Past, Present and Future. In *Stress and Tension Control. Stress Management 3*. F.G. MCGUIGAN., Sine, W.E. and J.M. Wallace (Eds.). Plenum Press. New York, 1989.
7. Cocke, J.A. Moynihan and N. Cohen, 1993. Exposure to conspecific alarm chemosignals allerts immune responses in BALB/C mice. *Brain Behaviour and Immun.*, 4: 36-46.
8. Ben-Eliyahu, S., 2003. The promotion of tumour metastasis by surgery and stress: Immunological basis and implications for psychoneuroimmunology. *Brain Behav. Immun.*, 17: 27-36.
9. Bauar, J.R.A.R., C. Mcneil, E. Trentelman, S.A. Swift and J.D. Mason, 1978. The depression of T. lymphocyte after traving. *The American J. Surgery*, 136: 674-680.
10. Romeo, C., A. Cruccetti, A. Turiaco, P. Impellizzeri, N. Turiaco, C. Di Bella, M.V. Merlino, S. Cifala, M. Basile, C. Gentile and D.C. Salpietro, 2002. Monocyte and neutrophil activity after minor surgical stress. *J. Pediatr. Surg.*, 37: 741-4.
11. Dahanukar, S.A., U.M. Thatte, U.D. Deshmukh, M.K. Kulkarni and R.D. Bapat, 1996. The influence of surgical stress on the psychoneuro-endocrine-immune axis. *J. Postgrad. Med.*, 42: 12-4.
12. Dorian, B., P. Gartinkel, G. Brown, A. Shore, D. Gladman and E. Keystone, 1982. Aberrations in Lymphocyte sub-populations and functions during psychological stress. *Clinical and Experimental Immunology*, 50: 132-136.
13. Cohen-Cole, S., R. Cogen, A. Stevens, K. Kink, E. Gaitan, J. Hain and A. Freeman, 1981. Psychological, endocrine and immune factors in acute, necrotizing ulcerative gingivitis ("Trenchmouth"). *Psychosomatic Medicine*, 43: 91-95.
14. Pauly, J.L., J.F. Sokal and T. Han, 1971. Whole blood culture technique for functional studies of lymphocyte reactivity to mitogens, antigens and homologous lymphocytes. *J. Laboratory and Clinical Medicine*, 82: 500-512.
15. Rosemurgy, A.S., E.E. Zervos, J.A. Sweeney and J.Y. Djeu, 1997. Candida antigen titre dilution and death after injury. *Br. J. Surgery*, 84: 771-774.
16. Reed, S.G. and P. Scott, 1993. T-cell and cytokine responses in Leishmaniasis. *Current Opinion in Immunology*, 5: 524-531.

17. Sadick, M.D., F.T. Hoinzel and B.J. Holaday, 1990. Care of murine Leishmaniasis with anti-interleukin 4 monoclonal antibody. *J. Exptl. Med.*, 171: 115-127.
18. Danek, I.A., M.S. O'Dorisco, M. O'Dorisco and J.M. George, 1983. *J. Immunol.*, 131:173.
19. Barry, M.C., C.M. Condon, R.W. Watson, H.P. Redmond, M. El Jack, R.G. Watson and B. Hayes, 1997. Pre-operative neutrophil and monocyte activation state predicts post-operative neutrophil and monocyte function. *Eur. J. Surg.*, 163: 739-45.
20. Esposito, S., 2001. Immune system and surgical site infection. *J. Chemotherapy*, 1:12-6.
21. Brand, J.M., P. Schmucker, T. Breidhardt and H. Kirchner, 2001. Upregulation of IFN-gamma and soluble interleukin-2 receptor release and altered serum cortisol and prolactin concentration during general anesthesia. *J. Interferon. Cytokine Res.*, 21: 793-6.
22. Maruna, P., R. Gurlich, R. Frasko and I. Chachkhiani, 2000. Postoperative immunosuppression-a physiological process and source of complications. Minireview. *Rozhl. Chir.*, 79: 589-95.