

Inflammatory response in open and laparoscopic cholecystectomy



Ann. Ital. Chir., LXXII, 6, 2001

G. Di Vita, C. Sciumè, S. Milano, R. Patti,
G. Lauria Lauria, G. Di Bella, R. Ca ruso,
P. Leo, E. Cillari

Università degli Studi di Palermo
I Divisione di Chirurgia Generale
Direttore: Prof. P. Leo
Dipartimento di Biopatologia e Metodologie Biomediche
Azienda Ospedaliera V. Cervello Laboratorio di Patologia
Clinica
Direttore: Prof. E. Cillari

Introduction

Laparoscopic cholecystectomy (LC) has become the gold standard technique over the open procedure for the treatment of the non- neoplastic disease of the gallbladder [1, 2]. Advantages of LC over the traditional open cholecystectomy (OC) are small skin incision, less pain, more rapid post- operative recovery associated with a reduced postoperative morbidity, early return to normal activity [3, 4]. The exact mechanism explaining the benefit of minimally invasive LC is unclear. In the last years, several studies have been published indicating as possible causes of the favourable clinical outcome of LC the reduction of neuroendocrin stress and acute phase response, associated with a better preservation of immune response [5-9]. After OC and LC has been observed a significant increment of interleukin (IL-6) and C-reactive protein (CPR) compared with the basal values [3, 5, 6, 10-18]. In many studies, comparing the two types of operation, a significant increase of IL-6 and CPR has been showed in patients that were underwent to OC [5, 10-13]; others, studies do not point out differences about IL-6 [3, 14-17] and CPR [6, 14, 16]. The data about modifications of other markers of acute phase are few [11,19]. In this study the modifications of IL-6, CPR, ceruloplasmin, alpha 1 antitrypsin, fibrinogen, transferrin, albumin and leukocytes counts after OC or LC have been evaluated.

Abstract

The modifications of IL-6, CRP, ceruloplasmin, alpha 1 antitrypsin, fibrinogen, transferrin, albumin and leukocytes counts have been evaluated after traditional open cholecystectomy (OC) or laparoscopic cholecystectomy (LC). Forty-two patients were included in this study, 20 underwent to OC and 22 underwent to LC. Serum samples were performed before surgery and at distance of 6, 24, 48 and 168 hours. The results show a more significant increase in acute phase inflammatory response after OC compared with LC as attested by highest values of leukocytosis, IL-6, CRP, fibrinogen and alpha 1 antitrypsin and lower levels of albumin. In conclusion, after LC, the phase acute response is attenuate and it can explain the reduced period of convalescence of patients treated with LC.
Key words: IL-6 and acute phase proteins in cholecystectomy.

Riassunto

LA RISPOSTA INFIAMMATORIA NEI PAZIENTI SOTTOPOSTI A COLECISTECTOMIA LAPAROSCOPICA

Sono state valutate, dopo colecistectomia laparotomica (OC) e laparoscopica (LC) le modificazioni dei seguenti parametri: interleuchina (IL)-6, proteina c reattiva (CRP), ceruloplasmina, alfa 1 antitripsina, fibrinogeno, transferrina, albumina e conta leucocitaria. Lo studio è stato condotto su 42 pazienti, 20 sottoposti ad OC e 22 ad LC. I campioni ematici sono stati eseguiti prima dell'intervento ed a distanza di 6, 24, 48 e 168 ore. I risultati mostrano un significativo incremento della risposta di fase acuta nei pazienti sottoposti ad OC rispetto a quelli LC come dimostrano gli alti valori di leucocitosi, IL-6, CRP, fibrinogeno, alfa 1 antitripsina, ed i bassi livelli di albumina. L'attenuata risposta di fase acuta dopo LC può spiegare il ridotto periodo di convalescenza dei pazienti trattati con tale metodica.
Parole chiave: IL-6, proteine di fase acuta, colecistectomia.

Pervenuto in Redazione il 13 Novembre 2000

Lavoro eseguito con i fondi della Ricerca Scientifica del Ministero dell'Università e della Ricerca Scientifica e Tecnologica (M.U.R.S.T.) 1996-98.

Ann. Ital. Chir., LXXII, 6, 2001 669

Materials and methods

Patients

Forty-two patients (age range 25-60 years) with symptomatic gallstone disease were included in this study. All of them were without gallbladder complications (acute cholecystitis, choledocholithiasis), metabolic, endocrine, hepatic or renal diseases. Furthermore, nobody assumed steroid, neither nonsteroid anti-inflammatory drugs, or received any transfusion. One group of patients was under the care of a surgeon of the team of the first Division of Surgery Department from University who routinely carried out LC (n. = 22) and the other under the cure of surgeon of the second Division of the same Surgery Department who performed OC (n. = 20). Patients gave written informed consent and the local ethics committee approved the study. Preoperatively, all patients received enoxaparin sodium (20 mg) and antibiotic prophylaxis (Ig of intramuscular cefuroxime). Both study groups received the standard anaesthetic procedures using thiopental sodium for induction, vecuronium for neuromuscular blockade, isoflurane and fentanyl citrate for analgesia. OC were carried out through an 8 cm subcostal incision. LC by the four trocars techniques [20] using a CO₂ pneumoperitoneum maintained at 12-14 mmHg. Patients were classified as either grade I, II or III according to American Society of Anaesthesiologists (ASA) grading system [21]. Age, gender, anesthesiologic grading, duration of operation and body weight index are given in Table I. LC patients left the hospital 2-3 days after the operation, whereas OC patients 4-5 days after surgery. Peripheral venous bloods samples were collected 24 hours prior to surgery and than 6, 24, 48 and 168 hours postoperatively.

Cell counts

Blood counts were performed by using an ADVIA 120 (Bayer Diagnostic, Munich, Germany) which performed absolute numbers and percentages of leukocyte count.

Table I – PATIENTS CHARACTERISTICS

	OC	LC
Age (years)	47 ± 15	52 ± 25
Gender (f/m)	14/6	18/4
Body mass index (Kg/in 2)	26 ± 5	24 ± 7
Anesthesiologic Grading (ASA)		
I	6	8
II	8	6
III	6	6
Duration of operation (min)	62 ± 28	66 ± 30

Data are expressed as mean ± standard deviation P > 0.05 all parameters.

Samples and Assay

Samples were from blood within 30 min of venipuncture by clotting and centrifugation at 400g for 10 min at rt. Samples were aliquoted and frozen at - 70°C until assayed.

For IL-6 determination available ELISA Kits were used (Euro Clone, Ltd, UK).

The principle of the assays was sandwich enzyme immune assay which utilises monoclonal antibody (MoAb) immobilised on a solid phase to capture antigen from the test specimen, and a peroxidase - conjugated monoclonal added to bind the antigens captured by the first antibody, as previously used [22].

CRP, transferrin, alpha 1- antitrypsin and ceruloplasmin plasma levels were determined by nephelometric analysis. Commercially available Kits were purchased from Behring, Vienna, Austria. Fibrinogen was determined using the method of Clauss and Kits from Behring, Vienna, Austria. Albumin was determined using biochemical analyser.

Statistical analysis

Standard deviation (SD) and standard error (SE) were calculated and statistical significance analysed by Student's t-test. Variance analysis was determined by ANOVA. Spearman rank correlation test was used to correlate different variables. Differences between two groups of patients were calculated by the Mann Whitney U test. The Wilcoxon signed rank test was used to evaluate paired data. P<0.05 was considered statistically significant.

Result

Age, gender, anesthesiologic grading, duration of operation and body weight index were no significant difference between the two groups (Tab. I) All patients showed an uncomplicated intra and post-operative course.

Interleukin-6

IL-6 levels were significantly increased in both groups compared their baseline levels (Tab. II). In patients who underwent OC, IL-6 began to significantly increase immediately after the operation with a peak 6 hours after operation. Its level remained significantly high (P<0.01) 48 hours after surgery, declining to the preoperative level 7 days afterwards. Also in the LC group patients IL-6 levels were significantly increased overtime compared with the baseline level, but it was significantly (P<0.01) lower than that in the OC group. Furthermore, in LC patients, after the IL-6 peak at hour 6, a marked decrease was observed by day 1.

Table II – EVALUATION OF IL-6 MODIFICATIONS AFTER OPEN CHOLECYSTECTOMY (OC) OR LAPAROSCOPIE CHOLECYSTECTOMY (LC).

Time	OC	LC
Preop.	5.3 ± 2.3	2.3 ± 0.6
+6h	39.1 ± 5.1**	14.6 ± 2.5**
+24h	37.2 ± 4.5**	8.7 ± 2.9*
+48h	31.5 ± 5**	8.5 ± 2.5*
+168h	4.2 ± 2.2	3.5 ± 2.1

Data are expressed as mean ± standard deviation.

Serum levels in health control group (pg/ml) 0.8±0.9.

Significance versus preoperative value *P<0.05; **P<0.01.

Leukocytes

The analysis of leukocyte counts in OC and LC groups are shown in Fig. 1a. As indicated cholecystectomy determined a significant leukocytosis 6-h, 1 and 2 days postoperatively in both groups. This increase was significantly higher in OC than in LC patients. OC was associated with a significant increase in the percentage and in the absolute number of neutrophils, with the contemporary reduction in lymphocytes (data not show). LC patients showed a significant (P<0.01) leukocytosis. Furthermore, LC subjects had a significant increase (P<0.01) in the absolute number of lymphocytes, even though their percentage values were not modified. (data not show). Monocytes were not significantly altered in both groups.

C-reactive protein

CRP levels were significantly increased in both groups compared their baseline levels (Fig. 1b). In patients who underwent OC, CRP began to significantly increase immediately after the operation with a peak between 24 and 48h, declining to the preoperative level 7 days afterwards. Also in the LC group patients CRP levels were significantly increased overtime compared with the baseline level (P<0.05), but they were significantly (P<0.05) lower than those observed in the OC group 24-48 h after surgery.

Transferrin and ceruloplasmin

For both groups all values of transferrin and ceruloplasmin were within normal range and failed to show any changes postoperatively (data not shown). There was no difference when preoperative values were compared to post operative data.

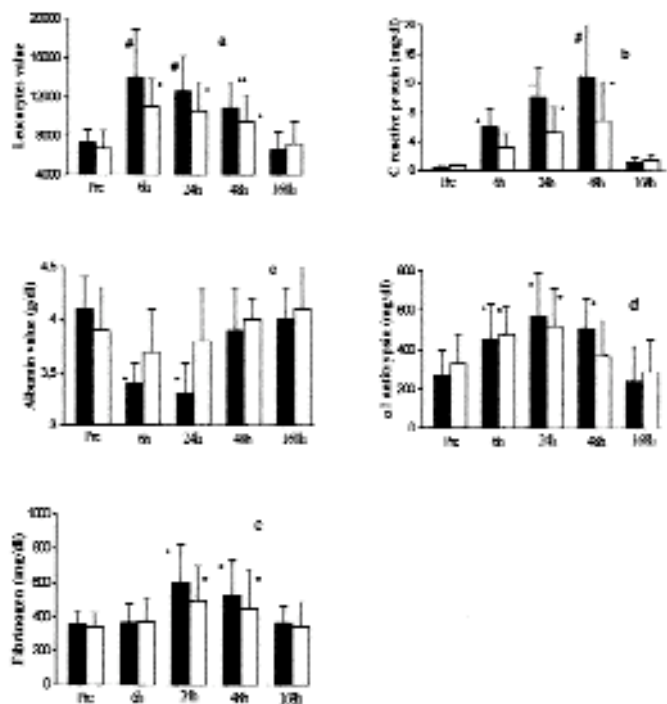


Fig.1: Leukocytes count (a) and serum level of C reactive protein (b), albumin (c), alpha 1 antitrypsin (d) and fibrinogen (e) after traditional open cholecystectomy ■ or laparoscopic cholecystectomy □. Significance versus preoperative values. *P<0.05; **P<0.01; #P<0.003; °P<0.002.

Albumin

In patients that were subjected to LC treatment plasmatic levels of albumin didn't undergo significative difference versus basal values. After OC albumin's level were reduced at 6 h and 24 h (P<0,05) (Fig. 1c).

Alpha 1 antitrypsin

Alpha 1 antitrypsin levels were significantly increased in both groups compared their baseline values at 6h and 24 h. In LC the values were normal at 48 h, while in OC were significantly increased also at 48 h (Fig. 1d).

Fibrinogen

The circulating levels of fibrinogen resulted increased in post-operative phase in both groups, but a significant difference was noted only at 24 h and 48 h. At 168 h fibrinogen's levels were in normal range (Fig. 1e).

Discussion

The surgical operation is followed by activation of defence responses. These begin in the wound site with activation of leukocytes, fibroblasts and endothelial cells and they are cha-

racterised by leukocytosis and synthesis at hepatic level of specific proteins called acute phase proteins [23]; their concentration is proportional to surgical damage [24-26]. There is clinical evidence that the surgical insult, experienced by patients who undergone LC, differs significantly from that experienced by those undergoing OC, since LC is accompanied by less pain, better ventilatory function and almost total absence of secondary paralytic ileum (3, 4). Some of the benefits of minimal invasive surgery seem to be due to lower inflammatory response. Our study shows a more significant increase in acute phase inflammatory response after OC compared with LC as attested by highest values of leukocytosis, IL-6, CPR, fibrinogen, alpha 1 antitrypsin, and the lower levels of albumin. In our patients an immediate and relevant secretion of IL-6 was observed in the blood, according to previous report [3, 5, 6, 10-18]. One important function of IL-6 is to stimulate the production of acute phase proteins [27, 28] activating the complex inflammatory response. The high serum levels of IL-6 could be due to synthesis at the site of incision by infiltrating T cells [28-30]. Our observation of reduction of circulating lymphocytes, as well as the already described reduction of CD4+T cells [9] is consistent with this hypothesis. Other important sources of IL-6 could be the damaged endothelium and fibroblasts [28-30]. Since there is convincing evidence those neutrophils can release IL-6 [31, 32], the very high number of neutrophils could have a relevant role in causing high levels of IL-6. The high levels of IL-6 could be responsible of the increase in the blood of fibrinogen and CRP, as acute-phase proteins produced by the liver [33, 34]. Although recently it has been shown that monocytes are capable of inducing alpha 1 antitrypsin synthesis when stimulated by IL-6 [35] the liver seems to be the main site production in the immediate post-operative period [36]. The reduction of albumin's concentration was observed only in OC patients may be correct as consequence of a more generalised increase in microvascular permeability caused by circulating vasoactive inflammatory mediators [37, 38].

The reduced acute inflammatory response that was observed in patients underwent to LC can be due to the minimum tissue damage produced using this technique, probably due to the limited size of the laparotomy wound [19], or to the lesser intrabdominal tissue damage secondary to intraoperative manipulation [39], or to desiccation of exposed tissue [13], or to the lesser damage mesothelial peritoneal cells [40].

In conclusion after LC the phase acute response is attenuated and it can explain the reduced period of convalescence of patients treated with this surgical procedure.

References

- 1) Schirmer B.D., Edge S.B., Dix J., Hyser M.J., Hanks J.B., Jones R.S.: *Laparoscopic cholecystectomy. Treatment of choice for symptomatic cholelithiasis*. Ann Surg, 213:665-677, 1991.
- 2) Neugebauer E., Troidl H., Spangenberg W., Dietrich A., Lefering R.: *Conventional versus laparoscopic cholecystectomy and the randomized controlled trial*. Br J Surg, 78:150-154, 1991.
- 3) Berggren U., Gordh T., Grama D., Haglund U., Rastad J., Arvidsson D.: *Laparoscopic versus open cholecystectomy: hospitalisation, sick leave, analgesia and trauma responses*. Br J Surg, 81:1362-1365, 1994.
- 4) The Southern Surgeons Club: *A prospective analysis of 1518 laparoscopic cholecystectomies*. N Engl J Med, 324:1073-1078, 1991.
- 5) Karayiannakis A.J., Makri G.G., Mantzioka A., Karousos D., Karatzas G.: *Systemic stress response after laparoscopic or open cholecystectomy: a randomized trial*. Br J Surg, 84:467-471, 1997.
- 6) Redmond E.P., Watson R.W.G., Houghton T., Condron C., Watson R.G.K., Bouchier-Hayes M.Ch.: *Immune function in patients undergoing open vs laparoscopic cholecystectomy*. Arch Surg, 129:1240-1246, 1994.
- 7) Ueo H., Honda M., Adachi M., Inoue H., Nakashima H., Arinaga S., Akiyoshi T.: *Minimal increase in serum interleukin-6 levels during laparoscopic cholecystectomy*. Am J Surg, 168:358-360, 1994.
- 8) Griffith J.P., Everitt N.J., Lancaster F., Boylston A., Richards S.J., Scott C.S., Benson E.A., Sue-Ling H.M., Mc Maon M.J.: *Influence of laparoscopic and conventional cholecystectomy upon cell-mediated immunity*. Br J Surg, 82:677-680, 1995.
- 9) Kloosterman T., von Blomberg B.M.E., Borgstein P., Cuesta M.A., Scheper R.J., Meijer S.: *Unimpaired immune functions after laparoscopic cholecystectomy*. Surgery, 115:424-428, 1994.
- 10) Jakeways M.S.R., Mitchell V., Hashim J.A., Chadwick S.J.D., Shenkin A., Green C.J., Carli F.: *Metabolic and inflammatory responses after open or laparoscopic cholecystectomy*. Br J Surg, 85:127-131, 1994.
- 11) Bruce D.M., Smith M., Walker C.B.J., Heys S.D., Binnie N.R., Gough D.B., Broom J., Eremin O.: *Minimal access surgery for cholelithiasis induces an attenuated acute phase response*. Am J Surg, 178:232-234, 1999.
- 12) Bellon J.M., Manzano L., Larrad A., Hondurilla G.N., Bujan J., Alvarez-Mon M.: *Endocrine and immune response to injury after open and laparoscopic cholecystectomy*. Int Surg, 83:24-27, 1998.
- 13) Targarona E.M., Pons M.J., Balagué C., Espert J.J., Moral A., Martinez J., Gaya Filella X., Rivera F., Ballesta A., Trias M.: *Acute phase is the only significantly reduced component of the injury response after laparoscopic cholecystectomy*. World J Surg, 20:528-534, 1996.
- 14) Mc Mahon A.J., O'Dwyer P.J., Cruikshank A.M., Mc Millan D.C., O'Reilly D.S.J., Lowe G.D.O., Rumley A., Logan R.W., Baxter J.N.: *Comparison of metabolic responses to laparoscopic and minilaparotomy cholecystectomy*. Br J Surg, 80:1255-1258, 1993.
- 15) Thorell A., Nygren J., Essen P., Gutniak M., Loftenius A., Andersson B.: *The metabolic response to cholecystectomy: insulin resistance after open compared with laparoscopic operation*. Eur J Surg, 162:187-191, 1996.
- 16) Reith H.B., Kaman S., Mittelkotter O., Kilic Y., Kozushek W.: *Cytokine activation in patients undergoing open or laparoscopic*

cholecystectomy. Int Surg, 82:389-393, 1997.

17) Vander Velpen G., Penninckx F., Kerremans R., Van Damine J., Amout J.: *Interleukin 6 and coagulation fibrinolysis fluctuation after laparoscopic and conventional cholecystectomy*. Surg Endosc, 8:1216-1220, 1994.

18) Schietroma M., Riseti A., Carlei F., Maggi G., Cianca G., De Santis C., Centi D., Iannucci D., Recchia C.L., Gattucci S., Simi M.: *Risposta della interleukina-6 in pazienti sottoposti a colecistectomia laparoscopica o laparotomica*. Min Chir, 53:359-62, 1998.

19) Mealy K., Gallagher H., Barry M., Lennon F., Traynor O., Hyland J.: *Comparison of the physiological response to open and laparoscopic cholecystectomy*. Br J Surg, 79:1061-1064, 1992.

20) Grace P.A., Quereschi A., Coleman J., Keane R., Mc Entee G., Broe P., Osborne H., Bouchier-Hayes D.: *Reduced postoperative hospitalization after laparoscopic cholecystectomy*. Br J Surg, 78:160-162, 1991.

21) American Society Anesthesiologists: *New classification of physiology status*. Anesthesiology, 24:111, 1963.

22) Cillari E., Vitale G., Arcoleo F., D'Agostino P., Mocciano C., Gambino G., Malta R., Stassi G., Giordano C., Milano S., Mansueto S.: *In vivo and in vitro cytokine profiles and mononuclear cell subsets in Sicilian patients with active visceral leishmaniasis*. Cytokine, 7:740-745, 1995.

23) Baumann R., Gauldie J.: *The acute phase response*. Immunol Today, 15:74-80, 1995.

24) Dominioni L., Dionigi R., Cividini F.: *Determination of CRP and AAT for quantitative assessment of surgical trauma*. Eur Surg Res, 12:133-136, 1980.

25) Colley C.M., Fleck A., Goode A.W., Muller B.R., Myers M.A.: *Early time course of acute phase protein response in man*. J Clin Pathol, 36:203-207, 1983.

26) Steel D.M., Whitehead A.S.: *The major acute phase reactants: C-reactive protein, serum amyloid P component and serum amyloid A protein*. Immunol Today, 15:81-87, 1994.

27) Stadnyk A.W., Gauldie J.: *The acute phase protein response during parasitic infection*. Immunol Today, 12:7-12, 1991.

28) Ivashkiv L.B.: *Cytokine expression and cell activation in inflammatory arthritis*. Adv Immunol, 63:337-376, 1996.

29) Grau G.E.: *Implications of cytokines in immunopathology: experimental and clinical data*. Eur Cytokine Netw, 1:203-210, 1990.

30) Van Snick J.: *Interleukin-6: an overview*. Ann Rev immunol, 8:253-278, 1990.

31) Chakravorty D., Kumar K.S.: *Interaction of lipopolysaccharide with human small intestinal lamina propria fibroblasts favors neutrophil migration and peripheral blood mononuclear cell adhesion by the production of proinflammatory mediators and adhesion molecules*. Biochim Biophys Acta, 1453:261-272, 1999.

32) Cassatella M.A.: *The production of cytokines by polymorphonuclear neutrophils*. Immunol Today, 16:21-26, 1995.

33) Marinkovic S., Jahreis G.P., Wong G.G., Baumann H.: *IL-6 modulates the synthesis of a specific set of acute phase plasma proteins in vivo*. J Immunol, 142:808-812, 1989.

34) Rock C.S., Coyle S.M., Keogh C.V., Lazarus D.D., Hawes A.S., Leskiw M., Moldawer L.L., Stein T.P., Lowry S.F.: *Influence of hypercortisolemia on the acute-phase protein response to endotoxin in humans*. Surgery, 112:467-474, 1992.

35) Perlmutter D.H., May L.T., Sehgal P.R.: *Interferon B2/interleukin-6 modulates synthesis of alpha-1-antitrypsin in human mononuclear phagocytes and in human hepatoma cells*. J Clin Invest, 84:138-144, 1989.

36) Wortel C.H., van Deventer S.J.H., Aarden L.A., Lygidakis N.J., Buller H.R., Hoek F.J., Horikx J., Ten Cate J.W.: *Interleukin-6 mediates host defense responses induced by abdominal surgery*. Surgery, 114:564-570, 1993.

37) Fleck A., Colley C.M., Myers M.A.: *Liver export proteins and trauma*. Br Med Bull, 41:265-273, 1985.

38) Le J., Vilcek J.: *Biology of disease. Interleukin 6: a multifunctional cytokine regulating immune reactions and the acute phase proteins response*. Lab Invest, 61:588-602, 1989.

39) Shimada M., Wffichurch R.A., Beloucif S., Robotharn J.L.: *Effects of anesthesia and surgery on plasma cytokine levels*. J Crit Care, 8:109-113, 1993.

40) Grablowitz V., Schippers E., Schumpelinck V., Klosterhalven B., Bittinger F.: *Quantification of peritoneal mesothelial cell damage after open vs laparoscopic cholecystectomy*. Surg Endosc, 7:228-233, 1993.

Commento

Commentary

Prof. Emanuele LEZOCHE
Ordinario di Chirurgia
Università degli Studi "La Sapienza"

Il lavoro è molto interessante e rappresenta un altro importante contributo per comprendere meglio la qualità di vita nel paziente sottoposto a colecistectomia laparotomica e laparoscopica.

Il valore scientifico di questo articolo è notevole e mostra chiaramente che il sistema immunologico non subisce danni significativi con la chirurgia laparoscopica, contrariamente a quanto accade con la chirurgia laparotomica dove si verificano alterazioni dei parametri immunologici anche in un semplice intervento come la colecistectomia.

This paper is very interesting and it represents another significant contribution to the comprehension of the different quality of life in patient undergone laparoscopy versus open cholecistectomy. The scientific value of the paper is remarkable and it clearly shows that the immunological system is not significant impaired by the laparoscopic surgery. On the contrary open surgery also in an easy operation, as cholecistectomy, induces significant change of the immunological parameters.

Autori corrispondenti:

Prof. Enrico CILLARI
Azienda Ospedaliera V. Cervello
Via Trabucco, 180
90146 PALERMO
Tel.: 091-6374973
Fax: 091-6885343
e-mail: enrico.cillari@iol.it

Prof. Gaetano DI VITA
Via Autonomia Siciliana, 70
90143 PALERMO
Tel.: 091-3629619
Fax: 091-6552771
e-mail: gaediv@tin.it

