Pakistan J. Zool., vol. 45(5), pp. 1207-1213, 2013

Circulatory Proteins in Women with Breast Cancer and their Chemotherapeutic Responses

Tahzeeb Fatima, Nabila Roohi* and Rabia Abid
Department of Zoology, University of the Punjab, Quaid-i-Azam Campus, Lahore-54590, Pakistan

Abstract.- Study concerns the analysis of serum total proteins and fractions (albumin, total globulins, gamma and non-gamma globulins) of breast cancer patients following chemotherapy (FAC regimen). For this purpose, blood samples of 84 breast cancer patients were collected from Institute of Nuclear Medicine and Oncology, Lahore (INMOL) before and after chemotherapy. These were compared with 52 age and sex matched healthy volunteers. Analysis of serum total proteins and fractions were carried out using commercially available kits by semi-automated clinical chemistry analyzer. Results were analyzed, statistically, using one-way analysis of variance and Tukey's post hoc multiple comparison test. Serum total proteins have shown a non-significant increase in patients when compared with controls. A remarkable reduction in serum albumin level ($p<0.0001$) was observed in newly diagnosed patients with a non-significant recovery of this protein following chemotherapy. Comparatively, serum total globulins, gamma globulins and non-gamma globulins levels have shown significant increase ($p<0.0001$, $p=0.0104$ and $p<0.0001$, respectively) in newly diagnosed patients showing a non-significant recovery following chemotherapy. Menopausal status and surgery did not show any role in changing serum protein profiles. Our results provide preliminary information about total serum proteins and fractions of breast cancer patients indicating lowered serum albumin and higher serum globulins to compensate albumin reduction, in patients. Chemotherapy did not play a significant role in recovery of these proteins.

Key words: Serum protein profile, breast cancer, chemotherapy.

INTRODUCTION

Breast cancer is the commonest malignancy of females all over the world and second leading cause of death due to cancer among females (Mahmood *et al.*, 2006). All women regardless of their racial or ethnic origin or heritage are at risk of developing breast cancer (Hunter *et al.*, 2000). Worldwide, breast cancer is the most frequent cancer after nonmelanoma skin cancer (Gonzalez-Angulo *et al.*, 2007). The human serum protein levels are often elevated in breast cancer patients. Total human serum protein profile is made up of albumin and globulins (DeGowin *et al*., 2004). The traditional method for measuring total protein uses the Biuret reagent. This measurement is usually performed on automated analyzers along with other laboratory tests (Gates and Fink, 2008).

Human serum albumin (HSA) is the most abundant plasma protein constituting about 50-60% of total serum protein (TSP) **(**Zunszain *et al.*, 2003)**.** Through a number of studies, a striking decrease in albumin levels has been observed in breast cancer patients and often used as prognostic indicator in these patients (Al-Joudi, 2005). The term globulin encompasses a heterogeneous group of proteins with typical high molecular weight, and both solubility and electrophoretic migration rates lower than for albumin (Marieb and Hoehn, 2010). In response to reduced levels of serum albumin, in breast cancer patients, the albumin to globulin ratio is lowered due to an increase in globulins, mainly immunoglobulins synthesized by lymphocytes to compensate for the reduced serum albumin. The failure of these cells to raise globulins to levels that are high enough to compensate for the reduced albumin may indicate advanced disease, where protein synthesis is reduced and/or protein catabolism has accelerated (Pisters and Brennen, 1990; Inui, 2002). These lower levels of serum globulins could be used as a supplementary parameter in determining the prognosis of patients with advanced cases of colorectal and breast cancers and is also an indication of immunosupression in these patients (Al-Joudi, 2005).

In many countries, mammography is used as a population-based screening method in breast cancer patients older than 50 years. Although breast

* Corresponding author: nabilaruhi@gmail.com
0030-9923/2013/0005-1207 $ 8.00/0

cancer mortality rate has decreased over the last decade (Jemal *et al.,* 2009), mainly because of its early detection through widespread mammography screening (Etzioni *et al.,* 2003), the specificity of the method is relatively low and it may lead to over diagnosis. Furthermore, up to 20% of new breast cancer incidents are not detected by this X-ray method, and for younger women with a genetic background of breast cancer, the sensitivity is not more than 40% (Brennan *et al.,* 2009).

Treatment options of breast cancer include surgical, cytotoxic, hormonal, and chemotherapeutic agents and sometimes radiation therapy (Gonzalez-Angulo *et al.,* 2007). FAC regimen is a combination of three different types of drugs, 5-fluorouracil (5-FU), adriamycin and cyclophosphamide. Adriamycin in combination with cyclophosphamide and 5-fluorouracil is superior to other types of regimen administration (Martin *et al.,* 2003). Among these fluorouracil is an antimetabolite, acting either by competing for binding sites on enzymes or through a direct incorporation into DNA or RNA and thus interfering with cell growth and proliferation. Adriamycin is an antibiotic, while cyclophosphamide is an alkylating agent capable of introducing alkyl groups into nucleophilic sites on other molecules through the formation of covalent bonds (Craig and Stitzel, 2006). The present study was conducted to check and evaluate the changes in serum protein profile of newly diagnosed breast cancer patients, if they could play any significant role in diagnosis of tumor. The second aim of the study was to investigate the effects of FAC regimen on recovery of altered serum protein profile.

MATERIALS AND METHODS

Patients and normal subjects

Ethical Review Committee (ERC) of Institute of Nuclear Medicine and Oncology (INMOL) Lahore approved the study. Initially, 117 newly diagnosed breast cancer patients were recruited for the study, out of which 96 patients fulfilled the criteria and were selected as subjects of the study. The participation rate was 100% among the patients. Out of these, 12 died within three months of the measurements of their serum proteins and 84 patients completed the follow-up for more than three months. They were grouped according to their menopausal status *i.e.* premenopausal (n=33) and postmenopausal (n=51). Control subjects were randomly selected as a reference population for comparative purpose and were also grouped as premenopausal (n=28) and postmenopausal (n=24). They were all healthy, non-pregnant, non-smokers and without any previous history of chronic disease. All of the participants belonged to the same socio-economic status and age groups (26-63 years). The participation rate was 74% in controls (n=52). The objectives of the study were explained to each woman and a written consent was obtained.

A detailed clinical history for each of the patient was recorded on a proforma designed for the study. History of pregnancy, usage of oral contraceptives, hormonal medications, surgery and use of other chemotherapeutic drugs was recorded.

Women with pregnancy, hypertension, diabetes mellitus, amenorrhoea, cardiovascular disease, smoking or those on any form of drug therapy were excluded from the study. The blood samples of each of the patients were obtained before chemotherapy and 20 days following five courses of chemotherapy.

Study protocol

The women were interviewed and examined for the signs and symptoms of breast cancer. The diagnosis was based on clinical history, general physical examination, mammography and histological measurements for cell differentiation. According to the data recorded from each patient file, all breast cancer patients were of the histological grades 2 and 3 (WHO, 1981).

Laboratory analysis

Protein analyses were carried out on serum samples from 84 breast cancer patients and 52 controls**.** Blood samples were processed to obtain serum after centrifugation at 3000rpm for 15 minutes and were preserved at -70°C before further examination. Both control and patient's samples were analyzed for total proteins and albumin using commercially available kits (Chema Diagnostica, Italy). On the other hand, serum gamma globulins were analyzed by precipitating these from serum

with ammonium sulphate-sodium chloride reagent (Wolfson *et al.,* 1948). Biuret reagent was used to estimate gamma globulins in these precipitates. The quantitative determination was carried out using clinical chemistry analyzer (Model 5010, Robert Riele GmbH & Co. KG. D-13467, Berlin, Germany). Total serum globulins were determined by subtracting the values of albumin from total protein and the non-gamma globulins were measured by subtracting the values of gamma globulins from globulins for each patient.

Statistical analysis

Statistical analysis was made by using GraphPad Prism version 5.00 (San Diego California, USA). Data was presented as Mean ± SEM, analyzed statistically using one-way ANOVA and Tukey's post hoc multiple comparison tests and employed in comparing the variations amongst the cohort of the study. Differences were considered to be significant at $p < 0.05$.

RESULTS

Serum protein profile including total proteins, albumin, globulins, gamma globulins and non-gamma globulins of control, newly diagnosed patients, and those undergoing chemotherapy were determined. Inter group comparisons were made to assess the variations.

Total proteins

An overall comparison of TSP levels of healthy subjects, newly diagnosed breast cancer patients and the patients undergoing chemotherapy was performed. Non-significant alternations were observed amongst the comparable groups as indicated by one-way ANOVA (p=0.2480, CI: 95%). For multiple comparisons, Tukey's post-hoc analysis was performed. The average value of total proteins indicated a significant increase of 7% (CI: 95%, p=0.0496) in pre-treated patients when compared with control. There was a non-significant increase of 4% (CI: 95%, p=0.1227) and a non-significant decrease of 4% (CI: 95%, p=0.6613) in post-treated patients when compared with control and pre-treated group, respectively (Tables I, II).

Table I.- Average values (mean±SEM) of serum total proteins and its fractions (g/dl) in study groups.

	Control	Pre-treated	Post-treated
Total proteins	7.56±0.08	8.11±0.15	7.86±0.11
Albumin	4.44±0.05	3.56±0.06	3.61±0.11
Total globulins	3.12±0.08	4.55±0.15	4.21±0.15
Gamma globulins	2.59±0.06	2.98±0.07	2.74±0.06
Non-gamma globulins	0.51±0.04	1.57±0.14	1.51±0.19

Table II.- Percentage differences and 'p' value summary of serum total proteins and its fractions in comparable groups.

	Control vs Pre-treated	Control vs Post-treated	Pre-treated vs Post-treated
Total proteins	7↑ (p=0.0496)	4↑ (p=0.1222)	4↓ (p=0.6613)
Albumin	20↓ (p<0.0001)	19↓ (p<0.0001)	1↑ (p=0.8298)
Total globulins	45↑ (p<0.0001)	34↑ (p<0.0001)	8↓ (p=0.5439)
Gamma globulins	15↑ (p=0.0037)	5↑ (p=0.2903)	9↓ (p=0.3677)
Non-gamma globulins	207↑ (p<0.0001)	196↑ (p<0.0001)	5↓ (p=0.9046)

↑ Increase, ↓ Decrease
Values are significant at p< 0.05

Albumin

An overall comparison of serum albumin levels of healthy subjects, newly diagnosed breast cancer patients and the patients undergoing chemotherapy was performed. Highly significant alterations were observed amongst the comparable groups as indicated by one-way ANOVA (p<0.0001, CI: 99.9%). Following multiple comparisons, the average value of albumin indicated a highly significant decrease of 20% (CI: 99.9%, p<0.0001) in pre-treated patients when compared with control. There was a highly significant decrease of 19% (CI: 99.9%, p<0.0001) in post-treated patients when compared with control. However, albumin showed a non-significant increase of 1% (CI: 95%, p=0.8298) when post-treated patients were compared with pre-treated group (Tables I, II).

Total globulins

An overall comparison of serum total

globulin levels of healthy subjects, newly diagnosed breast cancer patients and the patients undergoing chemotherapy was performed. Highly significant alterations were observed amongst the comparable groups as indicated by one-way ANOVA ($p<0.0001$, CI: 99.9%). The average value of total globulins showed a highly significant increase of 45% (CI: 99.9%, $p<0.0001$) in pre-treated patients when compared with control. There was a highly significant increase of 34% (CI: 99.9%, $p<0.0001$) in post-treated patients when compared with control. However, the average value of total globulins showed a non-significant decrease of 8% (CI: 95%, $p=0.5439$) when post-treated patients were compared with pre-treated group (Tables I, II).

Gamma globulins

An overall comparison of serum gamma globulin levels of healthy subjects, newly diagnosed breast cancer patients and the patients undergoing chemotherapy was performed. Significant alterations were observed amongst the comparable groups as indicated by one-way ANOVA ($p=0.0104$, CI: 95%). The average value of gamma globulins indicated a highly significant increase of 15% (CI: 99%, $p=0.0037$) in pre-treated patients when compared with control. There was a non-significant increase of 5% (CI: 95%, $p=0.2903$) and a non-significant decrease of 9% (CI: 95%, $p=0.3677$) in post-treated patients when compared with control and pre-treated group (Tables I, II).

Non-gamma globulins

An overall comparison of serum non-gamma globulins levels of healthy subjects, newly diagnosed breast cancer patients and the patients undergoing chemotherapy was performed. Highly significant alternations were observed amongst the comparable groups as indicated by one-way ANOVA ($p<0.0001$, CI: 99.9%). The average value of non-gamma globulins was in pre-treated patients indicated a highly significant increase of 207% (CI: 99.9%, $p<0.0001$) when compared with control. There was a highly significant increase of 196% (CI: 99.9%, $p<0.0001$) in post-treated patients when compared with control. However, the average value of non-gamma globulins showed a non-significant decrease of 5% (CI: 95%, $P=0.9046$) in post-treated patients when compared with pre-treated group (Tables I, II).

DISCUSSION

The incidence of breast cancer in women has globally increased. Even in previously low-risk developing Asian countries, the incidence of breast cancer has increased sharply over the past three decades (Coleman, 2003). Despite the substantial progress made in cancer therapy, breast cancer is the second leading cause of female cancer deaths, following lung cancer (Jemal *et al.*, 2008).

Proteins are the most abundant molecules found in any cell, acting as a dynamic system with various biological functions and are generally considered together because of their common biosynthetic origin, participation in the same processes, and occurrence as major extracellular components of the circulatory system (Gorinstein *et al.*, 2000). The sequence of amino acids in a protein is defined by the sequence of a gene (Shepherd, 2009). These changes are translated at protein level where quantitative and qualitative modifications are found in tumor compared to normal samples. Similar to studies aimed at deciphering transcriptional changes important in cancer, proteomic approaches allow the global and comparative study of proteins in normal and pathological samples (Mathelin *et al.*, 2006). Some of the essential proteins have also been found to act as indicators of obesity (Akram *et al*., 2011), which in turn is a known risk factor of breast cancer and its relapse after treatment (Lorincz and Sukumar, 2006).

Although, occurrence of marked variations in different proteins is well known in malignancies, it is not the amount of total protein but the levels of different proteins that should be considered individually (DeGowin *et al*., 2004). This fact is supported by decrease in one type through catabolism (albumin) and increase in the other (globulins) through synthesis, in response to breast cancer to compensate the first which consequently contributes to the serum levels of total proteins (Al-Joudi, 2005). This could be the probable fact that supports a non-significant change in serum levels of total proteins in our study, where, no significant

change was noticed in the total protein concentration. HSA is the most abundant protein, comprising more than half of the blood serum proteins (Sokołowska *et al.*, 2009). Reduced serum albumin (SA) level is used as an independent prognostic indicator in several diseases, including cancer (Al-Joudi, 2005; Seve *et al*., 2006; Gupta *et al*., 2009). Our study indicated a marked reduction of 20% ($P<0.0001$) in HSA levels in pre-treated breast cancer patients as compared to their healthy counterparts. Such low levels of HSA are known to be associated with poorer survival and increased mortality of the patients regardless of the stage of breast cancer (Lis *et al*., 2003; Wheler *et al*., 2010).

Al-Joudi (2005) pointed out that a raise in serum globulin (G) levels always follows reduction in serum albumin in breast carcinoma. A similar trend showing an increase of 45% in serum globulins of pre-treated patients compared to 20% decrease in HSA has been observed in newly diagnosed breast cancer patients in present study. However, in advanced metastatic stage, a partial compensation of globular proteins to albumin has been observed showing the failure of lymphocytes to raise globulins to the levels that are high enough to compensate for lowered albumin levels in serum. This is because in metastatic stage the protein synthesis is reduced and/or protein catabolism has accelerated. This implies that the reduction in HSA is further aggravated by the failure of G to compensate (Al-Joudi and Wahab, 2004; Al Joudi, 2005).

A significant increase of 15% in serum gamma globulins (immunoglobulins) of pre-treated breast cancer patients in present investigation suggests a secondary defense reaction against increasing tumor load. The increased levels of serum immunoglobulins associated with the patients of carcinoma breast with metastasis have led to conclude that these levels can lead to better assessment of the staging of carcinoma breast and thereby its management (Saxena, 1993; Alsabti, 2006).

Non-gamma fraction of globulins constitutes a large number of proteins mainly classified as α1, α2 and β globulins. Our study indicated a highly significant increase in non-gamma fraction of globulins, in breast cancer patients when compared to control. Many of these proteins have been indicated to play a possible role in etiology of breast cancer (Enriori *et al*., 1986; Nachtigall, 1999; Thompson *et al*., 2006; Weinstein *et al*., 2006).

FAC is an anthracycline-based chemotherapy, administered in doses of different concentration. The main goal of the breast cancer therapy is to prolong the survival where life expectancy of older women is frequently viewed as limited most probably due to a number of combined illnesses (Hunter *et al*., 2000). Despite of higher efficacy of adriamycin (Bonadonna *et al*., 2004), methotrexate is more frequently administered to older and sicker women, possibly because of higher risk of anthracycline-induced toxicities in these patients (Kadakia *et al*., 2013). Also, FAC has proven to be less cost effective than other adjuvant chemotherapies available for breast cancer patients (Lee *et al*., 2009; Mittmann *et al*., 2010).

The present study indicated that treatment with FAC regimen did not restore serum levels of any fraction of total protein significantly, where most of our patients were above 50 years of age. These results shows that failure of FAC in improving serum protein profile could possibly result in adverse outcomes including anthracycline related cardiovascular diseases and mortality. However, as the information provided by these results is preliminary, using the latest and advanced techniques in proteomics, it is possible to identify and sequence the specific proteins that alter in breast cancer patients following chemotherapy in our population. As proteins are the essential components of human body normal metabolism, there is a need of using a combination of drugs other than FAC, or changing the concentration of each drug in present dose which could help improving serum protein profile and overall survival in these patients. Using adjuvant therapies other than FAC could end up with a good economic value in adjuvant settings and protection against anthracycline-induced toxic outcomes, especially in older patients. In addition, while recommending chemotherapy, physicians are suggested to remain aware of the possible potential of getting chemotherapy associated complications during a long-term follow-up.

The increase in non-gamma fraction of total protein in our study could be due to the increase in

potential biomarker proteins for breast cancer. It is therefore suggested to expand this study at transcriptional and translational level, analyzing non-gamma fraction of protein to trace novel markers that can improve both sensitivity and specificity for early detection of breast cancer where the results of mammography are inconclusive.

Although our findings suggest several practical implications, the small sample size being studied is a limitation of our study. Therefore, a large-scale population study should be conducted for a comparative analysis to study effectiveness of different adjuvant therapies available for breast cancer that could result in better prognosis.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge all the participants of this study who agreed to give their blood samples to be used in the investigation. Major source of funding for the present investigation came from the Department of Zoology, University of the Punjab, which is gratefully acknowledged

Declaration of no conflict interest

There are no conflicts relating to the article.

REFERENCES

AKRAM, A.M., ALI, R., ROOHI, N. AND ILYAS, A., 2011. Quantitative profiling of serum protein fractions in young obese human. *Pakistan J. Zool.*, **43:** 489-495.

AL-JOUDI, F.S. AND WAHAB, N.A., 2004. The utilization of an index for serum globulin compensation in diseases associated with decreased serum albumin. *Med. J. Malaysia,* **59:** 495-501.

AL-JOUDI, F.S., 2005. Prognostic value of an index for serum globulin compensation in colon and breast cancers. *Singapore med. J.*, **46:** 710-713.

ALSABTI, A.K., 2006. Serum immunoglobulins in breast cancer. *J. Surg. Oncol.*, **11:** 129-133.

BONADONNA, G., ZAMBETTI, M., MOLITERNI, A., GIANNI, L. AND VALAGUSSA, P., 2004. Clinical relevance of different sequencing of doxorubicin and cyclophosphamide, methotrexate, and fluorouracil in operable breast cancer. *J. clin. Oncol.*, **22:** 1614-1620.

BRENNAN, M.E., HOUSSAMI, N., LORD, S., MACASKILL, P., IRWIG, L., DIXON, J.M., WARREN, R.M. AND CIATTO, S., 2009. Magnetic resonance imaging screening of the contralateral breast in women with newly diagnosed breast cancer: systematic review and meta-analysis of incremental cancer detection and impact on surgical management. *J. clin. Oncol.*, **27:** 5640–5649.

COLEMAN, R.E., 2003. Current and future status of adjuvant therapy for breast cancer. *Cancer,* **97:** 880-886.

CRAIG, C.R. AND STITZEL, R.E., 2006. *Modern pharmacology with clinical applications*. 6th ed., Lippincott Williams & Wilkins Publishers, pp. 509-638.

DEGOWIN, R.L., LEBLOND, R.F. AND BROWN, D.D., 2004. *DeGowin's diagnostic examination.* 8th ed., McGraw Hill Professional Publishers, pp. 948-959.

ENRIORI, C.L., ORSINI, W., CREMONA, M.C., ETKIN, A.E., CARDILLO LR. AND REFORZO-MEMBRIVES, J., 1986. Decrease of circulating level of SHBG in postmenopausal obese women as a risk factor in breast cancer: Reversible effect of weight loss. *Gynecol. Oncol.*, **23:** 77-86.

ETZIONI, R., URBAN, N., RAMSEY, S., MCINTOSH, M., SCHWARTZ, S., REID, B., RADICH, J., ANDERSON, G. AND HARTWELL, L., 2003. The case for early detection. *Nat. Rev. Cancer,* **3:** 243-252.

GATES, R.A. AND FINK, R.M., 2008. *Oncology nursing secrets*. 3rd ed., pp. 523. Westline Industrial Drive St. Louis, Missouri Publishers.

GONZALEZ-ANGULO, A.M., MORALES-VASQUEZ, F. AND HORTOBAGYI, G.N., 2007. Overview of resistance to systemic therapy in patients with breast cancer. *Adv. exp. Med. Biol.*, **608:** 1-22.

GORINSTEIN, S., GOSHEV, I., MONCHEVA, S., ZEMSER, M., WEISZ, M., CASPI, A., LIBMAN, I., LERNER, H.T., TRAKHTENBERG, S., MARTÍN-BELLOSO, O., 2000. Intrinsic tryptophan fluorescence of human serum proteins and related conformational changes. *J. Prot. Chem.,* **19:** 637-642.

GUPTA, D., LAMMERSFELD, C.A., VASHI, P.G., DAHLK, S., GRUTSCH, J.F. AND LIS, C.G., 2009. Is serum albumin an independent predictor of survival in ovarian cancer? *Ovar. Cancer,* **2:** 52-56.

HUNTER, C.P., JOHNSON, A. AND MUSS, H.B., 2000. *Cancer in the elderly*. Marcel Dekker Inc. Madison Avenue, New York, pp. 236-243.

INUI, A., 2002. Cancer anorexia-cachexia syndrome: current issues in research and management. *CA Cancer J. Clin.*, **52:** 72-91.

JEMAL, A., SIEGEL, R., WARD, E., HAO, Y., XU, J. AND THUN, M.J., 2009. Cancer statistics, 2009. *CA Cancer J. Clin.*, **59:** 225-249.

JEMAL, A., SIEGEL, R., WARD, E., HAO, Y., XU, J., MURRAY, T. AND THUN, M., 2008. Cancer statistics, 2008. *CA Cancer J. Clin.*, **58:** 71–96.

KADAKIA, A., RAJAN, S.S., SUSAN, A., DU, X.L. AND JOHNSON, M.L., 2013. CMF-Regimen preferred as first-course chemotherapy for older and sicker women with breast cancer: findings from a SEER-medicare-based population study. *Am. J. clin. Oncol.*, Publish

Ahead of Print, post author corrections, 19 April 2013.

LEE, S.G., JEE, Y.G., CHUNG, H.C., KIM, S.B., RO, J., IM, Y.H., IM, S.A. AND SEO, J.H., 2009. Cost-effectiveness analysis of adjuvant therapy for node positive breast cancer in Korea: docetaxel, doxorubicin and cyclophosphamide (TAC) versus fluorouracil, doxorubicin and cyclophosphamide (FAC). *Breast Cancer Res. Treat.*, **114:** 589-595.

LIS, C.G., GRUTSCH, J.F., VASHI, P.G. AND LAMMERSFELD, C.A., 2003. Is serum albumin an independent predictor of survival in patients with breast cancer? *J. Parent. Enter. Nutr.*, **27:** 10-15.

LORINCZ, A.M. AND SUKUMAR, S., 2006. Molecular links between obesity and breast cancer. *Endocrine-Related Cancer*, **13:** 279-292.

MAHMOOD, S., RANA, T.F. AND AHMAD, M., 2006. Common determinants of Ca Breast–a case control study in Lahore. *Annls. King Edward med. Coll.*, **12:** 227–228.

MARIEB, E.M. AND HOEHN, K., 2010. *Human anatomy & physiology*. 8th ed., Pearson Benjamin Cummings, San Francisco, CA, pp. 636.

MARTIN, M., VILLAR, A., SOLE-CALVO, A., GONZALEZ, R., MASSUTI, B., LIZON, J., CAMPS, C., CARRATO, A., CASADO, A., CANDEL, M.T., ALBANELL, J., ARANDA, J., MUNARRIZ, B., CAMPBELL, J. AND DIAZ-RUBIO, E., 2003. Doxorubicin in combination with fluorouracil and cyclophosphamide (i.v. FAC regimen, day 1, 21) versus methotrexate in combination with fluorouracil and cyclophosphamide (i.v. CMF regimen, day 1, 21) as adjuvant chemotherapy for operable breast cancer: a study by the GEICAM group. *Annls. Oncol.*, **14:** 833-842.

MATHELIN, C., TOMASETTO, C., CROMER, A. AND RIO, M.C., 2006. Proteomics and breast cancer. *Gynecol. Obstet. Fertil.*, **34:** 1161-1169.

MITTMANN, S., VERMA, M., KOO, K., ALLOUL AND TRUDEAU, M., 2010. Cost effectiveness of TAC versus FAC in adjuvant treatment of node-positive breast cancer. *Curr. Oncol.*, **17**: 7–16.

NACHTIGALL, L.E., 1999. Sex hormone-binding globulin and breast cancer risk. *Primary Care Update for OB/GYNS.*, **6:** 39-45.

PISTERS, P.W. AND BRENNEN, M.F., 1990. Amino acid metabolism in human cancer cachexia. *Annu. Rev. Nutr.*, **10:** 107-132.

SAXENA, S.P., MISHRA, V.K., BASU, P.K., PRAKASH, O. AND SHARMA, S.P., 1993. Significance of serum immunoglobulin levels in conglomeration with trans-sternal phlebography in the phlebography management of breast cancer. *Indian J. Pathol. Microbiol.*, **36:** 21-27.

SEVE, P., RAY-COQUARD, I., TRILLET-LENOIR, V., SAWYER, M., HANSON, J., BROUSSOLLE, C., NEGRIER, S., DUMONTET, C. AND MACKEY, J.R., 2006. Low serum albumin levels and liver metastasis are powerful prognostic markers for survival in patients with carcinomas of unknown primary site. *Cancer*, **107:** 2698-2705.

SHEPHERD, GM., 2009. *Creating modern neuroscience: The revolutionary 1950s*. Oxford University Press, pp. 24.

SOKOŁOWSKA, M., WSZELAKA-RYLIK, M., POZNAŃSKI, J. AND BAL, W., 2009. Spectroscopic and thermodynamic determination of three distinct binding sites for Co(II) ions in human serum albumin. *Inorg. Biochem.*, **103:** 1005-1013.

THOMPSON, D.K., HADDOW, J.E., SMITH, D.E. AND RITCHIE, R.F., 2006. Elevated serum acute phase protein levels as predictors of disseminated breast cancer. *Cancer Cytopathol.*, **51:** 2100-2104.

WEINSTEIN, P.S., SKINNER, M., SIPE, J.D., LOKICH, J.J., ZAMCHECK, N. AND COHEN, A.S., 2006. Acute-phase proteins or tumour markers: The role of SAA, SAP, CRP and CEA as indicators of metastasis in a broad spectrum of neoplastic diseases. *Scand. J. Immunol.*, **19:** 193-198.

WHELER, J., TSIMBERIDOU, A.M., MOULDER, S., CRISTOFANILLI M., HONG, D., NAING, A., PATHAK, R., LIU, S., FENG, L. AND KURZROCK, R., 2010. Clinical outcomes of patients with breast cancer in a phase I clinic: The M. D. Anderson Cancer Center experience. *Clin. Breast Cancer*, **10:** 46-51.

WOLFSON, W.Q., COHN, C., CALVAARY, E. AND ICHIBA, F., Studies in serum proteins. 1948. Rapid procedure for estimation of total proteins, true albumin, total globulins, alpha globulins, beta globulins and gamma globulins in 1.0 mL of serum. *Am. J. clin. Path.* **18:** 723-730.

WORLD HEALTH ORGANIZATION, 1981. *Histological typing of breast tumours*: *International Histological Classification of Tumours* No.2. Ed. 2, World Health organization, Geneva: Switzerland, pp. 19.

ZUNSZAIN, P.A., GHUMAN, J., KOMATSU, T., TSUCHIDA, E. AND CURRY, S., 2003. Crystal structural analysis of human serum albumin complexed with hemin and fatty acid. *BMC Struct. Biol.*, **3:** 1186-1472.

(Received 25 June 2012, revised 15 July 2013)