# The Effect of Aging on Cryglobulin responses in Pulmonary Tuberculosis Patients

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## ABSTRACT

In the present work attempts were, made to verify the hypothesis that aging may suppress cryoglobulin responses among pulmonary tuberculus patient .

Two main groups of test pulmonary tuberculus patients one with age group of $\geq 60$ years (aged) and the other with age group of $\leq 55$ years (non aged) patients. These two groups were equaly subdivided into males and females subgroups. Apparently healthy normal subjects were elected as control with same age groups as test patients. The test and control groups were subjected to study of Cryoglobulinemia responses in accordance with Irish guide line for study of Cryoglobulinemia.

The  aged patients of $\geq 60$ years old showed low Cryocrit percent, low CRP levels and high RF level, C3, C4 and IgA in comparison to non aged $\leq 55$ years old patients which  showed  high Cryocrit percent, high CRP levels and low RF level and IgA levels.

This Cryoglobulinemia associated is secondary to Pulmonary Tuberculosis and of mixed IgG-IgA-IgM types.

Cryocrit percent, RF, C3,C4 and Igs class determination proved to be valid battery of tests for immunodiagnosis of Cryoglobulinemia and for testing the effect of aging.

## الخلاصة

في هذه الدراسة محاولة لتحقيق مرضية لكون الشيخوخة تثبط استجابة الكلوبيولين المناعي البارد في مرضى التدرن الرئوي.

استخدمت زمرتين رئيستين من مرضى التدرن الرئوي احداهما شيوخ بعمر ٦٠ سنة اوكثر والاخرى مرضى تدرن رئوي بعمر ٥٥ سنة او اقل وجرى تقسيم كل من هاتين الزمرتين بالتساوي الى زمر ثانوية من ذكور واناث كما وجرى اختيار افراد احصاء باعمار مشابه باعمار المرضى بوصفها زمرة سيطرة . خضعت كل من زمر الاختبار والسيطرة الى التحري عن زيادة كلوبين البارد.

تبين بان الشيوخ قد اظهروا نسبة كرايوكرت واطئة ، بروتين طور حاد واطئ ومستويات عالية من C3 و C4 و IgA و RF ، بالمقارنة بينت زمرة ٥٥ سنة ( غير شيوخ) نسبة كرايوكت عالية بروتين طور حاد عالي وعامل رثوانية واطئ وكانت زيادى الالكلوبيولين البارد من النوع المختلط IgM –IgA– IgG . وبهذا فان زيادة الالكلوبيولين البارد ممكنة الاستخدام لتحري عن الشيخوخة في مرضى التدرن الرئوي.

**INTRODDUCTION**

Aging has been subdivided into early (55-65) years, middle (65-85) years and late (85-99) years. Although it is the extreme of the humane life span (centenarians $\geq$ 100 years). In this stage of life span, however, human undergoes many behavioral and functional changes (Clark,1999). To explain what is going on during senility, several mechanisms have been put forward like gene regulation theory (Plectcher *et al*,2002; Weidruch *et al*,2002; Krikwood,2002); Cell senescence, telomere theory (Blackourn.2000); Accumulation of damage (Goldsmith,2008). Antagonistic paleography (Goldsmith,2008). Disposable soma theory (Goldsmith,2008); And programmed cell death theory (Mitteldoff,2006). Thus many changes are known to occure during aging like histological, anatomic, physiologic and immunologic changes (Clark.1999). The immunologic, however, They may involves T, B cell function, Complement levels as well as acute phase proteins in addition to autoimmune response (Carson,1996; Casals *et al*,2003; Bruunsgaard,2006). Mean while, the informations on the changes in case of Cryoglobulin responses, during aging appeared in litreature as scattered and sparsed cases with variable results (Casato *et al*,1993; Zingnego *et al*,2002; Abbas *et al*,2008). Pulmonary tuberculus in human was proved to be associated with secondary mixed cryoglobulinemia (Shnawa and Al-Gebori 2011) and since, no Systematic research programmed to uncovers the real effect of aging on Cryoglobulin responses, the present study was aimed to study the aging effects on Cryoglobulin responses among Pulmonary Tuberculosis Patients.

## MATEREALS AND METHODS

### Patients and Controls:.

Forty-eight Pulmonary Tuberculosis Patients were diagnosed (Aldamaluji,1972) at the respiratory disease center Hilla and enrolled in this study from Nov.10th April 2010 during data from 10/11/2009 to 23/4/2010. They were twenty-four males subdivided to $\geq$ 60 year and $\leq$ 55 years and Twenty-four females subdivided according to age in same manner as in males. Controls were matching same age groups of patients.

### Sampling and Processing:.

From each patients and control 5ml of blood without anticoagulant were collected and maintained at 37C° then Sera were obtained. Seven ml of Urine were taken from patients and Controls collects of clean catch Urine sampling.

2ml of patients and control sera were separately loaded into Sahli tube and processed in accordance with guidelines of Irish Committee of Cryoglobulinemia (Lynch,2006).

Urine sample (7ml amount) were filtered through whatman No1 filter paper, then processed for obtaining secretary urine globulin solution (Johnston & Thorpe,1982with modification of Shnawa & Mahdi,2004). Two mls of secretary urine globulin solution were loaded in Sahli tube and processed for Cryoglobulinemia in accordance with guidelines of Irish Committee (Lynch,2006).

### The characterization of Cryoglobulin :.

Rheumatoid Factor and acute phase proteins C were tested by direct Qualitative and Semi-quantitative latex fixation in accordance with manufacture instruction.

Immunofixation studies for C3, C4, IgG, IgA and IgM were done by Radial Immunodiffusion in gel containing anti C3, antiC4, anti IgG, anti IgA and anti IgM (Mancini *et al*,1965).

### Statistical Analysis :

Mean, Median, Range, percentages, LSD and graphing were made using SSPS programme (Wester *et al*,2008).

**RUSELTS**
Cryoproteins:

Cryoproteins preparations were separated from patient sera and urine patients these were of six types. They included; Cryglobulins, IgM anti IgG autoantibodies, the RF, acute phase proteins C, C3 and C4 as showing in table1 to 6. Such proteins were predicted at 4 C° and dissolved at 37 or 45 C°. The physical nature of these cryoprecipctates were found different from one preparation of a patient to other. They appeared as gelatineous, crystalline or flocculent in serum preparations, while in urine preparations they appeared as colloidal yellowish white inn color. The ranges of time needed for the appearance of precipitates at 4C° were 12 to 120 hrs, though mostly appeared within 24 hrs. Similarly the time needed for re-dissolving were ranging from 1/2 to 3hrs cryoproteins were screened by cryocrite percentages (CP). CPs were found high in patients sera (4.334%) than the urine (3.416%) from same patients serum CP means for aged ≥ 60 years old were 3.5% while for the non-aged were 5.441%. The statistical differences between CP means of the aged as compared to non-aged male and female patients were significant there were marked. Individual variations in CP among patients, as indicated by median and range values as showing in table 1.

Cryo IgM anti IgG The RF;

Rheumated factors (RF) represent the autoimmune response. RF may be found both in the sera and urine of the patients 7:24 (14.85%). Or in the sera of patients only 6:48 (12.5 %) The RF titre means were 4.4 and 1.66 in sera and urine for non-aged patients, while for aged patient they were 6 and 2 in the sera and urine respectively. The RF concentrations means for the aged were 48 and 16 in sera and urine respectively. In comparison, they were 35.2 mg/ml and 13.33 mg/ml for sera and urine of non-aged patients as showing in table 2 .

Cryoacute phase patients C (CRP);

CRP were observed both in patients sera and urine as 5:48 (31.25%) and in patients sera only in a rate of 10:48 (20.85%). The CRP titre means in the sera and urine of the aged patients were 5.860 and 1.675 respectively as compared to non-aged patients they were 6.4 and 2.285 for patients sera and urine accordingly. The CRP concentration means in aged patients sera and urine were 35.2 and 11.25 mg/ml respectively. Though in non-aged patients sera and urine they were of 37.4 mg/ml and 13.214 mg/ml accordingly (table 3).

Cry complement components C3 and C4;

The serum Cryo C3 and C4 concentration means were higher than the reference values though C3 concentration means were high in aged than in non-aged patients as showing in table 4.

Cryoglobulins;

Three isotypes of cryoimmunoglobulins were determined as IgG, IgA and IgM in all patients sera. These cryoglobulins were either associated with RF or non RF associated. The concentration means of IgA were high in aged than in non-aged patients, whether they were RF or non RF associated. The RF association

in aged patients increases the IgG concentration means in those with non-RF association. Comparable IgG concentration means were noted in non-aged patients whether they were associated with RF or not as showing in table 5.

Cryoglobulinemia;

The noted type of cryoglobulinemia were mixed containing IgG-IgA-IgM. This cryoglobulinemia is either RF or non RF associated. It is secondary to pulmonary tuberculosis.

Aging and cryoglobulinemia;

Aging decreases CR, CRP & IGM levels, while it increases IgA, C3 and RF concentrations. Though it doesn't affects C4 levels in patients sera.

Table-1: Serum and Secretory Urinary Cryocrit percentage in aged Tuberculosis Patients:.

| Patients gender | Sample | Age | | | | | |
|---|---|---|---|---|---|---|---|
| | | Mean | | Median | | Range | |
| | | ≥ 60 yr | ≤ 55 yr | ≥ 60 yr | ≤ 55 yr | ≥ 60 yr | ≤ 55 yr |
| Male (12)* | Serum | 3.666 | 5.416 | 3.0 | 5.0 | 2-7 | 4-8 |
| | Urine | 2.833 | 4.33 | 3.0 | 4.0 | 1-5 | 3-7 |
| Female (12)* | Serum | 3.416 | 4.416 | 3.0 | 5.0 | 2-6 | 3-10 |
| | Urine | 2.583 | 3.916 | 3.0 | 4.0 | 1-4 | 3-6 |
| Total | Serum | 3.54 | 4.80 | 5.0 | 6 | 2-7 | 3-10 |
| | Urine | 2.48 | 4.12 | 3.0 | 1-55 | 3-7 | 3-7 |

* Patients Number

Table-2: RF Titer and RF Concentration in sera and urine aged and non-aged Tuberculosis Patients:.

| | | Age | Mean | Median | Range |
|---|---|---|---|---|---|
| Titer | Serum | (8)* ≥ 60 yr | 6 | 8 | 2-16 |
| | | (5)* ≤ 55 yr | 4.4 | 4 | 2-8 |
| | Urine | (4)* ≥ 60 yr | 2 | 1 | 1-4 |
| | | (3)* ≤ 55 yr | 1.66 | 2 | 1-2 |
| Concentration | Serum | Age | Mean | Median | Range |
| | | (8)* ≥ 60 yr | 48 | 32 | 16-128 |
| | | (5)* ≤ 55 yr | 35.2 | 32 | 16-64 |
| | Urine | (4)* ≥ 60 yr | 16 | 1 | 1-4 |
| | | (3)* ≤ 55 yr | 13.33 | 16 | 8-16 |

* Patients Numbers

Table-3: Cryo CRP Titer and CRP Concentration among aged and nonaged Tuberculosis Patients:.

| | | Age | Mean | Median | Range |
|---|---|---|---|---|---|
| Titer | Serum | (15)* ≥ 60 yr | 5.866 | 4 | 2-16 |
| | | (10)* ≤ 55 yr | 6.4 | 2 | 2-16 |
| | Urine | (8)* ≥ 60 yr | 1.875 | 2 | 1-4 |
| | | (7)* ≤ 55 yr | 2.285 | 4 | 1-4 |
| | | Age | Mean | Median | Range |
| Concentration | Serum | (15)* ≥ 60 yr | 35.2 | 24 | 12-96 |
| | | (16)* ≤ 55 yr | 37.4 | 12 | 12-96 |
| | Urine | (8)* ≥ 60 yr | 11.25 | 12 | 6-24 |
| | | (7)* ≤ 55 yr | 13.714 | 24 | 6-24 |

* Patients Numbers

Table-4: Serum Cryo C3 and C4 Concentrations of Pulmonary Tuberculosis aged and nonaged Patients:.

| Sex | Age | Sample | Mean | Median | Range |
|---|---|---|---|---|---|
| Male | ≥ 60 yr | C3 | 1.399 | 1.133 | 1.895 |
| | | C4 | 0.181 | 0.228 | 0.331 |
| | ≤ 55 yr | C3 | 1.266 | 1.133 | 1.332 |
| | | C4 | 0.231 | 0.228 | 0.331 |
| Female | ≥ 60 yr | C3 | 1.458 | 1.557 | 0.947 |
| | | C4 | 0.233 | 0.228 | 0.214 |
| | ≤ 55 yr | C3 | 1.094 | 1.133 | 0.829 |
| | | C4 | 0.240 | 0.228 | 0.429 |
| Total | ≥ 60 yr | C3 | 1.427 | 1.133 | 0.895 |
| | | C4 | 0.205 | 0.228 | 0.033 |
| | ≤ 55 yr | C3 | 1.186 | 1.133 | 1.332 |
| | | C4 | 0.205 | 0.228 | 0.456 |
| Reference Value | C3 | 0.86 ± 0.33 mg/ml | | | |
| | C4 | 0.11 ± 0.14 mg/ml | | | |

Table-5: Cryoimmunoglobulin class Concentrations among Tuberculosis Patients:.

| Sex | Age | Igs | Mean | Median | Range |
|---|---|---|---|---|---|
| Male | ≥ 60 yr | IgG | 13.718 | 13.418 | 16.503 |
| | | IgA | 4.350 | 3.546 | 8.949 |
| | | IgM | 1.233 | 1.406 | 1.471 |
| | ≤ 55 yr | IgG | 11.907 | 11.208 | 8.251 |
| | | IgA | 3.610 | 3.245 | 5.220 |
| | | IgM | 1.484 | 1.406 | 2.140 |
| Female | ≥ 60 yr | IgG | 12.092 | 11.208 | 13.261 |
| | | IgA | 4.597 | 4.997 | 4.195 |
| | | IgM | 1.320 | 1.696 | 1.471 |
| | ≤ 55 yr | IgG | 12.723 | 11.208 | 13.261 |
| | | IgA | 2.12 | 2.334 | 3.076 |
| | | IgM | 1.530 | 1.696 | 0.579 |
| Total | ≥ 60 yr | IgG | 12.995 | 11.208 | 16.502 |
| | | IgA | 4.466 | 4.944 | 8.949 |
| | | IgM | 1.274 | 1.696 | 2.141 |
| | ≤ 55 yr | IgG | 12.288 | 11.208 | 13..261 |
| | | IgA | 2.915 | 2.334 | 6.059 |
| | | IgM | 1.506 | 1.696 | 1.471 |
| Normal Value | | 0.08 mg/ml | | | |
| Mixed Croglobulinemia I | | 5.0 mg/ml | | | |
| Mixed Croglobulinemia II | | 1.0 mg/ml | | | |
| Mixed Croglobulinemia III | | Up to 25 mg/ml | | | |

Table-6: Mixed Cryoimmunoglobulin and RF associated among Tuberculosis Patients:.

| Patients groups | Age | Mean Igs Concentrations in mg/ml | | |
|---|---|---|---|---|
| | | IgG | IgA | IgM |
| RF associated Cryoglobulin | ≥ 60 yr | 15.824 | 5.006 | 1.350 |
| | ≤ 55 yr | 13.074 | 2.801 | 1.503 |
| Non RF associated Cryoglobulin | ≥ 60 yr | 11.576 | 4.001 | 1.261 |
| | ≤ 55 yr | 12.461 | 3.82 | 1.484 |
| Total | ≥ 60 yr | 12.995 | 4.465 | 1.233 |
| | ≤ 55 yr | 12.288 | 2.915 | 1.484 |
| Normal Value | 0.08 mg/ml | | | |
| Mixed Croglobulinemia I | 5.0 mg/ml | | | |
| Mixed Croglobulinemia II | 1.0 mg/ml | | | |
| Mixed Croglobulinemia III | Up to 25 mg/ml | | | |

## DISCUSSION

The separated cryoprotiens preparations as showing in the table 1-6 were of six types namely; Globulins, IgM anti IgG auto-antibodies, the RF, acute phase protein C, C3 and C4. Among which globulin in the optimal preparations is consistent with the own the characteristics of precipitate reversibility (Ferri, et.al., 2002; Sansonno and Dammaco, 2005. Shnawa and Algebori 2011) Shnawa and Algebori 2011 have proved its utility in mapping herd immunity of pulmonary tuberculus patients.

Aging was associated with an increase in IgA; C3 and RF levels and a decrease in CP, CRP, and IgM levels but it doesn't affect C4 values in patient. Thus CP, CRP, IgM, IgA, C3 and RF can be of use as test battery as showing in the tables 1-6 for checking the effect of aging on secondary cryoglobulinemia in pulmonary tuberculus patients.

Aging is , mostly associated with brook of tolerance, such a brook may mediate an autoimmune response (Brietbart and Stollar, 2000) . Tuberculosis however, may induced an autoimmune response by an epitope of M.tuberculosis that memicate a self endogenous epitope (Delves et. al.,2006). Thus there are three possibilities that might operate in namely aging alone, tuberculosis alone or aging and tuberculus disease could induce the auto reactive response represented by RF in these pulmonary tuberculus patients as showing in table 2 (Casals et. al. 2003, Carson, 1993).

The CRP levels in aged patients were lower that these of non-aged as showing in table 3 such low CRP values may be due to slowing secretion of CRP by hepatic cells as results of aging (Cesari et. al. 2004 Wester et. al., 2008) .

The complement component C4 was not affected by aging, while aging was associated with higher C3 values. Likewise, both of C3 as well as C4 values in patients were higher than the reference value as showing in table 4.

Such findings have been supported by the works of other in this context (Menzel et. al., 1999; Wilma et. al. ,2001 ; Plackett et. al. 2004).

IgG levels among aged patients (table 5) were high. Other workers (Cossarizza et. al.1997) have supports such observation but in normal immunoglobulin and not in cryoglobulins.

IgG concentration means were found higher in RF associated than in non RF associated patients (Cossarizza et. al., 1972). The reported cryoglobulinemia (table 5&6) was of mixed IgG-IgA-IgM type, secondary to pulmonary tuberculosis, can be classed II, III cryoglobulinemia (Dispenzier and Gorvic 1999; Morra, 2005 National Reference lab., 2009).

To hypothesize the possible mechanisms behind cryglobulin responses one may be of the opinion that a cryoactive immunodominate epitope(s) belongs to the agent M. tuberculosis infecting the host trigger immune cells to perform such cryo responses with an inherent tendency to cryoprecipitates (Haency, 1988).

Such features in (table 7) can be mediated by aging, chronic TB, as well as both. Aging and chronic intracellular M.tuberculosis infections (Kallemachillal and Gerovic, 1999; Dispenzier and Gerovic, 1999).

The major immune feature of cryoglobulinemia in both of the test patients groups are depicted in table 7.

Table-7: Major Cryoglobulinemia features :

| Feature | Non-aged | Aged |
|---|---|---|
| Nature | Secondary STB | Secondary SDB |
| CP | High | Low |
| Autoimmune Response RF | Low in positive cases | High in positive cases |
| Acute phase response C | High in positive cases | Low in positive cases |
| IgA | Low | High |
| C4 | Higher than Raf. Value equal to aged | As in non-aged equal to them |
| C3 | High | Higher |
| Mixed | IgG – IgA – IgM | Ig-G-IgM-high IgA |
| Response nature | Mucosal and systemic | Mucosal and systemic |

**REFRENCES**

Abbas OM ; Nabil A and Zeinab KH .2008. T (14;18) is Not Associated with Mixed Cryoglobulinemia or with Clonal B Cell Expansion in Egyptian Patients with Hepatitis C Virus Infection . Journal of the Egyptian Nat. Cancer Inst .20(2) : 149-157.

Al damaluji SF .1972. Tuberculosis For Medical Students and Practitioners in Iraq . 1<sup>th</sup> ed .William Heinemann medical Books Limited .

Blackburn EH .2000. Telomere states and cell fates. Nature .408 : 53-56.

Bruunsgaard H .2006. The clinical impact of systemic low-level inflammation in elderly populations . with special reference to cardiovascular disease , dementia and mortality. Danish Medical Bulletin .3 : 285-309.

Breitbart E and Stollar D .2000. Aging and the Human Immune system . IMAJ .2 : 703-707.

Casato M ; Vincent A ; Leopoldo PP ; Glenn BK ; Marco L ; Savino DV ; Cristina M ; Guido A ; Lorenzo B   .1997. Predictors of Long-Term Response to High-Dose Interferon Therapy in Type II Cryoglobulinemia Associated With Hepatitis C Virus Infection .The American Society of Hematology .90(10) : 3865-3873.

Carson DA .1993. Rheumatoid Factor. Textbook of Rheumatology (Eds. WN. Kelley ED. Harris, S. Ruddy, CB. Sledge), Saunders Comp. Philadelphia, London, Toronto, Tokyo pp : 155-164.

Casals MR ; Garc MC ; Brito MP and  Lopez AS .2003. Autoimmunity and geriatrics: Clinical Significance of Autoimmune manifestations in the elderly . Lupus . 12(5) : 341–355.

Clark WR .1999. A means to end. The biology basis of aging and death. Oxford University Press. Oxford.

Cesari M ; Pennix BW and Pahor M .2004. Inflammatory markers and physical performance in older persons: The InCHIANTI study. J Gerontol A Biol Sci Med Sci  .59 : 242-248.

Cossarizza A ; Ortolani C ; Monti D ; Franceschi C .1997. Cytometric analysis of immunosenescence . Cytometry .27 : 297–313.

Delves PJ ; Martin EJ ; Burton DR and Roitt IM .2006. Essential Immunology 11<sup>th</sup> . Blackwell Publishing, London.

Dispezieri A and Gorevic P .1999. Cryoglobulinaemia. Hematol Oncol Clin North Am .13(6) : 1315-49.

Ferri C ; Zignego AL and Pileri SA .2002. Cryoglobulins . J Clin Pathol .55 : 4–13.

Goldsmith TC .2008. Aging Theories and Their Implications for Medicine.tgoldsmith@azinet.com,www.azinet.com,aging.1:1-11.

Hayflanick L .1965. The limited in vivo life time of human diploid cell strains Exp Cell Res .37 : 614-636.

Haeney, M. 1988. Monoclonal Immunoglobulins. In French MAH (eds.) Immunoglobulin in health and disease MTP press limited, Boston, 168.

Johnston A and Thrope R .1982 . Immunochemistry in practice . p : 1-72 . Black well Scientific Puplication Oxford .

Kirkwood T .2002. Evolution of ageing. Mech. Ageing Dev .123 : 737– 45.

Kallemuchikkal U and Gorevic PD .1999. Evaluation of Cryoglobulins . Arch Pathol Lab Med .123 : 119-25.

Lynch PLM . 2006 . Audit of Cryoglobulin Determination in Northern Ireland . Clinical Biochemistry Department , Altnagelvin Area Hospital , Londonderry . Full implementation of all new guidelines : 1-13.

Mancini G ; Carbonara AO ; Heremans JF .1965. Immunochemical quantitative of antigen by single radial immunodiffusion . Immunochemistry .2 : 235-245.

Mitteldor f and Joshua .2006. Chaotic population dynamics and the evolution of ageing, Evolutionary Ecology Research .8 : 561–574.

Menzel EJ ; Zlabinger GJ ; DunkyA and Steffen C .1999. Autoimmunity and T-cell subpopulations in old age . Arch Gerontol. Geriatr .7 : 249–260.

Morra E .2005. Cryoglobulinemia . the American Society of Hematol : 368-372.

National Reference Laboratory .2009. Cryoglobulin, Qualitative with Reflex to IFE Typing and Quantitative IgA, IgG, and IgM . www.arupconsult.com .84108 : 800-801.

Plectcher SD ; Macdonald SJ ; Marguerie R ; Certa U ; Stearns SC ; Goldstein DB and Partridge L .2002. Genome-wide transcript profiles in aging and calorically restricted Drosophila melanogaster. Curr Biol .12 : 712-723.

Plackett TP ; Boehmer ED ; Fannce DE and Kovacs EJ .2004. Aging and innate immune cells . J leukoc Biol .76 : 291-299.

Sansonno D and Dammacco F .2005. Hapatitis C Virus , Cryoglobulinemia and Vasculitis : immune complex relation. Lancet Infect Dis .5 : 227-236.

Shnawa IMS and Mahdi MS .2004. Mucosal agglutination antibody to antigen of bacterial associated with urinary tract infection in human adults .Babylon University Journal .9 : 773-782.

Shnawa I.M.S. and ,Al-Gebori 2011. Secretory and circulatory secondaryCryoglobulinemu in Pulmonary Tuberculus Paticnts .Baby. Uni. J.Accepted.

Weindruch R ; Kayo T ; Lee CK and Prolla TA .2002. Gene expression profiling of aging using DNA microarrays. Mech Ageing Dev .123 : 177-193.

Wester LW ; Karl GB and Torgeir BW .2008. Is the concentration of C-reactive protein in bacteraemia associated with age . Immunity & Ageing .5 : 1-8.

Wilma CN ; Aline CM ; Adilson DC ; Luciana SH ; Carla LG and Roberto AF .2001. Immunologic Evaluation in Infective Endocarditis . Arq Bras Cardiol .76(1) : 48-52.

Zignego AL ; Clodoveo F ; Francesca  G ; Carlo G ; Patrizio C ; Monica M ; Maria M ; Elena DP ; Giorgio LV ; Giacomo L and Paolo G .2002. Prevalence of bcl-2 Rearrangement in Patients with Hepatitis C Virus–Related Mixed Cryoglobulinemia with or without B-Cell Lymphomas . Ann Intern Med .137 : 571-580.