

## Antibodies to p53 - can they serve as tumor markers in patients with malignant lymphomas?

Barbara Jezeršek<sup>1</sup> and Srdjan Novaković<sup>2</sup>

<sup>1</sup>Department of Medical Oncology, <sup>2</sup>Department of Tumor Biology,  
Institute of Oncology, Ljubljana, Slovenia

---

**Background.** Tumor suppressor gene p53 is mutated in approximately 21 % of patients with nonHodgkin's lymphomas (the percentage varying from 0 up to 67 % depending upon the histological type). Most of the mutations are point missense mutations resulting in nuclear accumulation of altered protein. Roughly one third of patients with overexpression of p53 protein develop circulating anti p53 antibodies. The present study was aimed at defining the usefulness of serial serological determinations of autoantibodies to p53 for clinical follow up of NHL patients.

**Patients and methods.** Serum levels of antibodies to p53 were determined in various time intervals in three lymphoma patients (who had elevated serum levels at the time of diagnosis) for maximum two years using the commercially available ELISA kit p53-Autoantikoerper ELISA 2. Generation.

**Results.** In all three cases the temporal patterns of anti p53 antibodies reflected accurately disease progression or regression, and even foretold a relapse ten months in advance. The reflection of disease regression by autoantibodies lagged approximately three months behind the morphological disappearance of the disease due to a long half life of the antibodies.

**Conclusion.** Our results confirmed the usefulness of antibodies to p53 as tumor markers for follow up of lymphoma patients, yet the subset of patients that could be appropriately followed up with this method is very limited due to the low proportion of patients that develop immune response to p53 protein.

*Key words:* lymphoma, non-Hodgkin; tumor markers, biological ; protein p53

---

### Introduction

p53 is a tumor suppressor gene the alterations of which are among the most frequent genetic changes detected in human neoplasms. Normal p53 acts as a "guardian of the genome" by preventing the proliferation of cells with damaged DNA. This function is achieved by the production of normal (wild type) p53 protein which acts on downstream

Received 19 April 2000

Accepted 21 May 2000

Correspondence to: Barbara Jezeršek, M.D., M.Sc.  
Dept. of Medical Oncology, Institute of Oncology,  
Zaloška 2, SI-1000 Ljubljana, Slovenia. Tel.: +386 1 323  
063 ext. 29 33, Fax: +386 1 4314 180, E-mail:  
snovakovic@onko-i.si

genes to arrest the cell cycle until the DNA damage is repaired, or if the damage is irreversible, to cause apoptosis.<sup>1,2</sup> The loss of wild type function usually occurs by a two-step mechanism comprising mutation of one copy of the p53 gene and deletion or inactivation of the remaining wild type allele. The outcome of the mutation is the synthesis of a protein with a changed conformation, a longer half life, and disordered function in terms of cellular growth.<sup>3</sup>

The incidence of p53 mutations in nonHodgkin's lymphomas (NHL) varies according to histological type and disease stage - it is high in aggressive lymphomas (Burkitt's and diffuse large B cell lymphomas) and lower in intermediate and indolent lymphomas. Regardless of the type - the incidence is generally higher in case of a relapse or after progression. Most of the mutations are missense mutations clustered in exons 5 to 8 of one p53 allele and are usually associated with deletion of the other allele, through 17p deletion.<sup>4</sup>

Different authors<sup>5-11</sup> reported that p53 protein can become immunogenic in various human tumors *in vivo*, indicating that alterations in the expression or properties of p53 associated with tumor development can be detected by screening the sera of cancer patients for anti p53 antibodies. The formation of autoantibodies to p53 is directed against two immunodominant regions located at the carboxy and amino termini of the protein outside the central mutational hot spot region<sup>7</sup> and is observed only in patients with p53 missense mutations that express detectable levels of p53 protein in their tumor cells.<sup>8</sup> The p53 specific immune response does not develop in cancer patients bearing stop, splice/stop, splice or frameshift mutations of p53 gene in tumor cells.<sup>8</sup>

According to the literature autoantibodies to p53 were identified in 2.6%<sup>10</sup> up to 21%<sup>6</sup> of the tested sera of NHL patients. In our previous study the percentages of p53 antibody

positive sera also varied greatly between different histological subtypes of NHL. In this paper we present a small study examining the usefulness of serial serological determinations of autoantibodies to p53 for clinical follow up of NHL patients.

## Materials and methods

### Patients

Three patients (two male and one female patient, ages 25, 64, and 47 years, respectively) with different types of NHL (REAL classification)<sup>12</sup> in which autoantibodies to p53 were detected at the time of diagnosis were followed up for maximum two years. In all three patients also p53 protein overexpression (indicating an underlying mutation of p53 gene) was identified immunohistochemically.

### Sample collection

Blood was collected in variable time intervals over a maximum two year period and sera were separated from the clot by centrifugation. The sera were then aliquoted and stored at -20°C until analysis. The samples were diluted in sample dilution buffer before assaying (so that the measurement results fell inside the area of a standard curve) and the determinations were executed according to manufacturer's instructions.

### Serum determinations

Serial determinations of autoantibodies to p53 were performed using the commercially available ELISA kit p53-Autoantikörper ELISA 2. Generation (Dianova, Hamburg, Germany). The assay is based on a sandwich technique with human recombinant p53 protein coated to the microtiter plate wells which binds the anti p53 autoantibodies of the

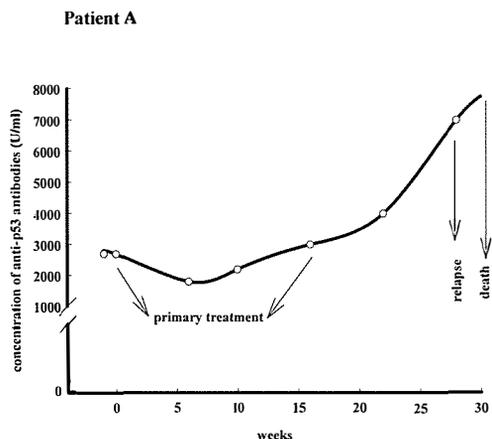
serum sample and a goat anti human IgG antibody (as the detection antibody) conjugated to peroxidase. The bound enzymatic activity is determined by addition of a chromogenic substrate and by measuring the resulting colored solution with a spectrophotometer. The concentration of the sample or the standard is directly proportional to the absorbance value measured.

## Results

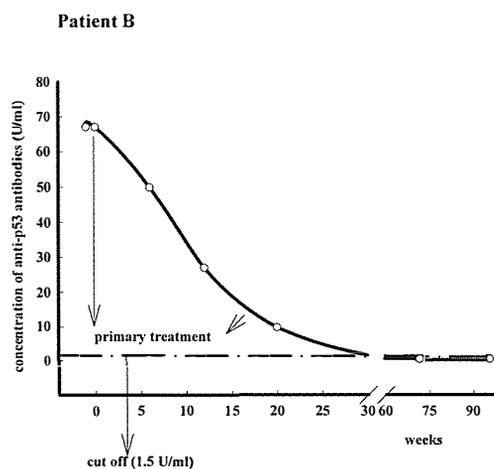
*Patient A* was diagnosed with Burkitt's lymphoma stage IV and was treated with aggressive chemotherapy that included high dose methotrexate, cyclophosphamide, ifosfamide, vincristine, adriamycin, etoposide, cytosine arabinoside and corticosteroids plus chemotherapeutic central nervous system prophylactic therapy according to BFM protocol. He responded well to primary treatment and there were no clinical signs of residual disease upon completion of the therapy. How-

ever, three months later he was admitted to the hospital with a massive relapse. Salvage chemotherapy was introduced, but with no effect, so the patient died of lymphoma shortly thereafter. The levels of antibodies to p53 were elevated prior to treatment, showed only a minor decrease during the primary treatment and started to rise before the primary treatment was completed. From there on the levels increased continuously reflecting the disease progression (Figure 1).

*Patient B* was diagnosed with an inoperable diffuse large cell B lymphoma of the stomach involving also paraaortic lymph nodes. He was treated with six cycles of CHOP regimen (cyclophosphamide, epidoxorubicin, vincristine, corticosteroids) plus radiation and achieved a complete remission. Surprisingly, during the two years of follow up there was no evidence of recurrence. Pretreatment levels of antibodies to p53 in this patient were elevated, but fell to normal (below the cut-off limit determined by the kit producer) as a consequence of successful treatment lagging approximately



**Figure 1.** Consecutive serum levels of autoantibodies to p53 protein in a patient with Burkitt's lymphoma reflected the dynamics of the disease. The patient responded well to primary treatment, but experienced a relapse (three months later) that was resistant to salvage therapy. The relapse was foretold by the increasing levels of autoantibodies to p53 protein already before the primary treatment was finished.



**Figure 2.** The downward trends in serum levels of autoantibodies to p53 protein confirmed the success of primary treatment in a patient with inoperable diffuse large cell B lymphoma of the stomach. The levels fell below the cut-off limit approximately three months after morphological disappearance of the disease.

two to three months behind the morphological disappearance of the disease (Figure 2).

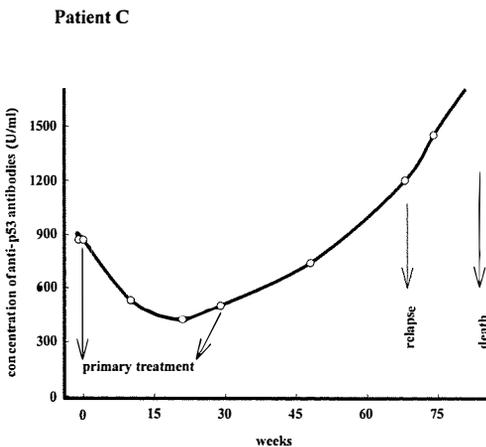
*Patient C* was diagnosed with follicle center cell lymphoma grade III and stage III. She was treated with ten cycles of CHOP regimen (cyclophosphamide, epidoxorubicin, vincristine, corticosteroids) plus radiation of the residuum after chemotherapy and with this combined treatment achieved a complete remission. Ten months later a relapse was detected and she was treated with salvage chemotherapy, but progressed during the treatment. Radiation therapy was applied to the bulky masses in the mediastinum - again with poor success, so the patient died of lymphoma shortly thereafter. The levels of antibodies to p53 were elevated prior to treatment and decreased somewhat reflecting the response to primary chemotherapy - but never reached the cut-off level. Two months upon completion of CHOP regimen the levels of antibodies to p53 started to rise again foretelling the relapse ten months before it was actually confirmed. During the salvage thera-

py there was a continuous increase in the antibody levels (Figure 3).

## Discussion

In contrast to various types of solid tumors (e.g. prostate cancer, breast cancer, colorectal cancer, germ tumors), in lymphomas there are no appropriate serum tumor markers for the easy follow up of the patient's disease status, with lactic dehydrogenase (LDH) and beta-2-microglobulin being the best approximations to the definition of a serum lymphoma tumor marker.<sup>13</sup> However, in a certain proportion of NHL patients with overexpression of p53 protein in their tumor cells (due either to mutation of p53 gene or stabilization of the gene product by mechanism other than point mutation), this protein somehow becomes immunogenic, resulting in the production of autoantibodies to p53 protein.<sup>6,10</sup> It has been suggested that the appearance of such antibodies in the sera of cancer patients is a very early event - present already at the diagnosis.<sup>7,14</sup> The specificity of the autoantibodies to p53 protein is 100%<sup>11</sup>, but they are present in malignancy in general, and are not an indicator of the type of malignant disease. Therefore, the antibodies show some prerequisites for a good tumor marker also in some lymphoma patients. The frequency of autoantibodies' development, on the other hand, is relatively low, for example approximating only 25% of patients with hepatocellular carcinoma<sup>11</sup>, which is due to the facts that p53 is not mutated in every patient with malignant disease, and that the appearance of antibodies to p53 (protein) is not an obligatory consequence of p53 overexpression in tumor cells.

In our three cases the early presence of anti p53 antibodies, as well as their specificity, were confirmed, while only 8% of NHL patients developed an immune response to overexpressed p53 protein.



**Figure 3.** The serum levels of autoantibodies to p53 protein indicated the disease status in a patient with follicle center lymphoma grade III. The patient achieved a complete remission with the primary treatment, but relapsed ten months later and responded poorly to the salvage therapy. The relapse was foretold ten months in advance by the continuous increase in the serum levels of autoantibodies.

On the other hand, in accordance with observations of Angelopolou *et al.*<sup>10</sup> our results clearly demonstrated that the levels of anti p53 antibodies correlated well with the disease progression or regression, even foretelling the disease progression ten months before it was actually confirmed. In contrast to conventional tumor markers, which are known to have a shorter half life, the temporal patterns of antibodies reflecting tumor regression lagged approximately two to three months behind the morphological disappearance of the disease. This delay is explainable with the relatively long serum half lives of the antibodies, and the time required for the immune system to adapt to an altered immunological stimulus.

In conclusion, even though the existence of anti p53 antibodies in patients with NHL is a relatively rare event - namely, overexpression of p53 protein is found in approximately 21% of NHL (varying with the histological type and the antibody used in immunohistochemical methods)<sup>15-17</sup>, of which only a part develop an autoimmune response to p53 protein (for percentages see the Introduction) - these autoantibodies, when present, show the qualities of a good serum tumor marker and represent one of the simplest methods to follow up a subset of NHL patients. Moreover, due to the fact that the presence of antibodies to p53 protein in patients with different solid tumors was associated with more aggressive tumor types, shorter tumor free intervals and poor prognosis<sup>18,19</sup>, there is another strong argument speaking in favor of detecting antibodies to p53 protein in order to define the subset of patients being particularly at risk for an unfavorable outcome.

#### Acknowledgment

The technical assistance of Mrs. M Lavrič is gratefully acknowledged.

#### References

1. Jezeršek B, Novakovič S. p53 - the paradigm of tumor-suppressor genes? *Radiol Oncol* 1998; **32**: 373-83.
2. Lane DP. p53, guardian of the genome. *Nature* 1992; **358**: 15-6.
3. Hainaut P, Hollstein M. p53 and human cancer: the first ten thousand mutations. *Adv Cancer Res* 2000; **77**: 81-137.
4. Preudhomme C, Fenaux P. The clinical significance of mutations of the p53 tumor suppressor gene in haematological malignancies. *Br J Haematol* 1997; **98**: 502-11.
5. Crawford LV, Pim DC, Bulbrook RD. Detection of antibodies against the cellular protein p53 in sera from patients with breast cancer. *Int J Cancer* 1982; **30**: 403-8.
6. Caron de Fromentel C, May-Levin F, Mouriesse H, Lemerle J, Chandrasekaran K, May P. Presence of circulating antibodies against cellular protein p53 in a notable proportion of children with B-cell lymphoma. *Int J Cancer* 1987; **39**: 185-9.
7. Schlichtholz B, Legros Y, Gillet D, Gaillard C, Marty M, Lane D, et al. The immune response to p53 in breast cancer patients is directed against immunodominant epitopes unrelated to the mutational hot spot. *Cancer Res* 1992; **52**: 6380-4.
8. Winter FS, Minna JD, Johanson BE, Takahashi T, Gazdar AF, Carbone DP. Development of antibodies against p53 in lung cancer patients appears to be dependant on the type of p53 mutation. *Cancer Res* 1992; **52**: 4168-74.
9. Lubin R, Schlichtholz B, Bengoufa D, Zalcman G, Tredaniel J, Hirsch A, et al. Analysis of p53 antibodies in patients with various cancers define B-cell epitopes on human p53: distribution on primary structure and exposure on protein surface. *Cancer Res* 1993; **53**: 5872-6.
10. Angelopolou K, Diamandis EP, Sutherland DJA, Kellen JA, Bunting PS. Prevalence of serum antibodies against the p53 tumor suppressor gene protein in various cancers. *Int J Cancer* 1994; **58**: 480-7.
11. Volkmann M, Müller M, Hofmann WJ, Meyer M, Hagedorn J, Raeth U, et al. The humoral immune response to p53 in patients with hepatocellular carcinoma is specific for malignancy and independent of the  $\alpha$ -fetoprotein status. *Hepatology* 1993; **18**: 559-65.

12. Harris NL, Jaffe ES, Stein H, Banks PM, Chan JKC, Cleary ML, et al. A revised European-American Classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. *Blood* 1994; **84**: 1361-92.
13. Novaković S. *Pregled pomembnejših tumorskih označevalcev v klinični onkologiji*. Ljubljana: Onkološki inštitut; 2000.
14. Bennet WP, Colby TV, Travis WD, Borkowski A, Jones RT, Lane DP, et al. p53 protein accumulates frequently in early bronchial neoplasia. *Cancer Res* 1993; **53**: 4817-22.
15. Porter PL, Gown AM, Kramp SG, Coltrera MD. Widespread p53 overexpression in human malignant tumors. *Am J Pathol* 1992; **140**: 145-53.
16. Soini Y, Paakko P, Alavaikko M, Vahakangas K. p53 expression in lymphatic malignancies. *J Clin Pathol* 1992; **45**: 1011-4.
17. Said JW, Barrera R, Shintaku IP, Nakamura H, Koffler HP. Immunohistochemical analysis of p53 expression in malignant lymphomas. *Am J Pathol* 1992; **141**: 1343-8.
18. Houbiers JGA, van der Burg SH, van de Watering LMG, Tollenar RAEM, Brand A, van de Velde CJH, et al. Antibodies against p53 are associated with poor prognosis of colorectal cancer. *Br J Cancer* 1995; **72**: 637-41.
19. Peyrat JP, Bonnetterre J, Lubin R, Vanlemmens L, Fournier J, Soussi T. Prognostic significance of circulating p53 antibodies in patients undergoing surgery for locoregional breast cancer. *Lancet* 1995; **345**: 621-2.