Article

p53 Antibodies as a Diagnostic Marker for Cancer: a Metaanalysis

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Abstract: Importance: p53 is an unequivocal tumor suppressor altered in almost half of human cancers 1–4. P53 is auto-regulated by MDM2, an E3 ubiquitin ligase 5,6. Mice lacking MDM2 show embryonic lethality, while the double knockout of p53 and MDM2 can rescue the lethality 7. The p53 mutation in cancer (p53-mut) does not activate the

1. Introduction

P53 is an unequivocal tumor suppressor mutated in almost half of human cancers 1–4. P53 is auto-regulated by MDM2, an E3 ubiquitin ligase 5,6. Mice lacking MDM2 show embryonic lethality, while the double knockout of p53 and MDM2 can rescue the lethality 7. The p53 mutation in cancer (p53-mut) does not activate the
expression of the E3 ligase. Consequently, degradation of p53 protein is not down-modulated\[8\]. High expression of p53 by cells recapitulates in T-cells the production of antibodies against mutant or wild-type p53[8].

Prognostic biomarkers have a crucial role in medicine to measure the progression of a disease from samples of patients, such as metastasis in cancer, and they can aid clinicians to intervene with more precise medical interventions. In addition to the common notion that in humans loss of p53 increases genomic instability, stem-cell likeness, which ultimately leads to highly aggressive cancers, with invasive and metastatic properties. p53 antibodies (s-p53-Abs) are stably expressed in cancer patients serum and could have an important prognostic application. Many clinical studies have assessed in cancer patients the correlation between the expression of s-p53-Abs with tumor invasiveness grades, metastasis and prognosis.

Since 20-40% of p53-mut cancer patients have s-p53-Abs\[9\], we performed a meta-analysis of the current literature, investigating the role of s-p53-Abs as a prognostic factor and a predictor of response to anti-cancer treatments.

2. Material and Methods

The studies were identified according to the following inclusion criteria: 1) participants with cancer; 2) outcome results expressed in relation to the presence of a p53 antibody; 3) a primary outcome (disease free survival, overall survival or progression free survival) expressed as hazard ratio (HR). The following exclusion criteria were used: 1) insufficient data available to evaluate outcomes; 2) animal studies; 3) studies with less than 10 participants.

Two independent researchers revised the included studies, all disputes were evaluated with the corresponding author.

The summary estimates were generated using a fixed-effect model (Mantel–Haenszel method)[17] or a random-effect model (DerSimonian–Laird-method)[18] depending on the absence or presence of heterogeneity (I). A subgroup analysis was performed to highlight any differences between studies in terms of Overall Survival (OS), Disease Free Survival (DFS), Progression Free Survival (PFS), as summarized in Table 1.

When we used the keywords “p53 antibodies in early cancer”, “p53 antibodies in metastatic cancer”, “p53 antibodies impact on cancer progression”, the PubMed search yielded 1375 potentially relevant articles; studies as duplicates or reviews were excluded. After viewing the titles and abstracts of the 52 remaining studies, the full texts of 34 studies were retrieved and 12 studies [19–26] were included in the analysis (Table 1 and Table 2) as summarized in the flow chart of Figure 1.
Figure 1. Flowchart of literature research strategy.

Table 1. Clinical investigations of p53-wt antibodies in cancer. Main characteristics of clinical investigations for prognostic evaluation of serum p53-wt antibodies in cancer patients.
| Page | Patients | Inclusion | Surgery | Time | Notes |
|------|----------|-----------|---------|------|-------|
| 21   | 76 patients with transitional urinary bladder cell carcinoma. | S-p53-Abs ELISA, Antibodies for p53-wt | Surgery (TUR) + chemotherapy + radiotherapy (advanced stage) | 34 months | There was an association between the presence of s-p53-Abs and tumor p53 gene overexpression (p = 0.001). |
| 22   | 184 CRC patients. Dukes’ stage: A (n = 31); B (n = 84); C (n = 41); D (n = 28) | S-p53-Abs ELISA, Antibodies for p53-wt | Routine Biopsy | 96 months | p53-Abs correlated with shorter survival (0.02). |
| 23   | 170 CRC patients | S-p53Ab, CEA ELISA, Antibody for p53-wt | Surgery (resected tumour specimen) | 93.6 months (median value) | Positivity for s-p53Ab in CRC did not correlate with overall survival. Kaplan-Meier analysis revealed significant differences between patients with elevated s-p53Ab and CEA and those with levels of either or neither of these factors (p < 0.001). Conversely, Cox regression analysis revealed that a high level of CA19-9 was an independent prognostic factor for GC (hazard ratio (HR) = 3.864; 95% confidence interval (CI) = 1.248–11.959; p = 0.019). |
| 24   | 208 GC patients | S-p53Ab Detected with anti-p53 detection kit MESACUP anti-p53 Test Antibody for p53-wt | Surgery | 34 months | Did not observe a significant correlation between S-p53Abs and overall survival (hazard ratio = 2.052; 95% confidence interval (CI) = 0.891–4.726; p = 0.091). High levels of p53Abs correlated with worse survival compared to patients with lower levels of the antibodies (p = 0.02). |
| 25   | 231 SCLC patients | S-p53-Abs ELISA, Antibodies for p53-wt | Surgery Chemotherapy (227 out of 231 patients) | 3 months (at least) | Anti-p53 was not useful. |
| 26   | 80 HCC patients | S-p53-Abs | Inclusion: Cytohistologic - Percutaneous injection | 36 months | Anti-p53 was not useful. |
| Study | Patients | ELISA. Antibodies | Technical details | Diagnosis Details | Outcome | Conclusion |
|-------|----------|-------------------|------------------|------------------|--------|------------|
| 27    | 244 CRC patients | ELISA. Antibodies for p53-wt | Inclusion: preoperative CEA, CA-19-9, S-P53Ab. Primary tumour diagnosis | Surgery (colectomy plus lymph nodes dissection) | 33.8 months (median) | S-P53Ab had no power to predict the prognosis $p = 0.786$. Combined CEA and CA19-9 positivity was an exclusive independent prognostic factor $p = 0.034$. |
| 28    | 97 SCLC patients | ELISA. Antibodies for p53-wt | Inclusion: newly and proven diagnosed lung cancer | Bronchial biopsy | 18.1 months (median) | Patients with stage SCLC and p53-Ab had a median survival time of 10 months whereas limited-stage SCLC patients without p53-Ab had a median survival time of 17 months $p = 0.039$. |
| 29    | 133 esophageal squamous cell carcinoma (ESCC) patients | ELISA. Antibodies for p53-wt | Inclusion: histologically confirmed ESCC | Surgery | 36 months (median) | S-p53-Ab was detected in 39.1% (52 out of 133) of patients with ESCC, including 40.0% of patients with early-stage ESCC $p = 0.009$. The positivity for the TAA panel was independently associated with poor prognosis $P = 0.030$. |
| 30    | 1487 esophageal squamous cell carcinoma | ELISA. Antibodies for p53-wt | Inclusion: radical surgery with no neoadjuvant treatment | Esophagectomy | 42 months (median) | s-p53-Ab positivity was not significantly associated with overall survival $p = 0.049$. |
| 31    | 160 hepatocellular carcinoma | ELISA. Antibodies for p53-wt | Six hepatocellular carcinoma-associated antigens, including Sui1, p62, RalA, p53, NY-ESO-1, and c-myc antibodies by ELISA (TAA Panel) | Inclusion: histologically proven HCC | Surgery | The positivity of the TAA panel was independently associated with poor prognosis $p = 0.030$. |
Table 2. Clinical investigations of p53-mut antibodies in cancer. Main characteristics of clinical investigations for prognostic evaluation of serum p53-mut antibodies in cancer patients.

| Study Reference | Patients | Methods | Prognostic value of s-p53-Abs | Type of Study | Inference |
|-----------------|----------|---------|-------------------------------|---------------|-----------|
| [24]            | 111 gastric carcinoma patients | S-p53-Abs Levels of p53-mut were determined with a selective, quantitative ELISA kit | The survival time of serum-positive patients was significantly longer than that of patients with low/negative serum levels, with a survival rate of 41.2% and 14.9%, respectively, over 48 months (p < 0.05). | Retrospective | Significant correlation seen between levels of S-p53-mut Abs and patient survival rate |
| [25]            | 104 ovarian cancer patients | S-p53-Abs ELISA. Antibodies against p53K132Q (c.394A > C). | Overall survival (OS) was significantly higher for patients with antibodies to mutant p53 when compared with patients without p53 antibodies (P = .01). | Retrospective | OS is significantly increased in advanced stage ovarian cancer patients with antibodies to p53 |
134 lung cancer patients studied S-p53-Abs by Immunofluorescence. Antibodies against p53 R273H (c.818G > A) by ELISA. Presence of anti-p53 autoantibodies is almost exclusively linked to the presence of malignant disease. Retrospective study of presence of anti-p53 Abs had a significant correlation with shorter survival in NSCLC.

50 BC patients studied S-p53-Abs by ELISA. Antibodies against p53R273H (c.818G > A). s-p53-Abs were higher in BC patients with high risk vs. patients with low risk. The difference was not statistically significant (p = 0.15). Retrospective study of presence of s-p53 Abs showed higher risk for BC patients.

3. Results

A total of 2094 patients were included. The solid cancer patients were treated with adjuvant chemotherapy (such as cyclophosphamide, docetaxel, fluorouracil, epirubicin, methotrexate, vinorelbine), anti-HER2 (trastuzumab, pertuzumab or lapatinib), endocrine therapy (such as goserelin, tamoxifen), combination of these treatments, Herceptin, chemotherapy, nonsteroidal anti-inflammatory drug celecoxib, including radiotherapy or a surgical component in some cases (Table 1). The pooled analysis revealed that s-p53-Abs is a negative prognostic factor (HR: 1.48 [1.24, 1.77]; p<0.0001, Figure 2) in cancers. The analysis was performed using a random-effects model heterogeneity (I^2=19%).

![Figure 2](image)

**Figure 2. Metaanalysis of serum p53-antibodies.** The prognostic value of p53 antibodies in serum of cancer patients from eight clinical investigations was investigated in this metaanalysis.
The funnel plot (Figure 3) of the included studies showed symmetric funnel plot and no significant publication bias was identified.

![Funnel plot](image)

**Figure 3.** The funnel plot of included studies

4. **Discussions and conclusions**

The metanalysis showed that high levels of p53 antibodies significantly correlated with worse clinical outcomes. Our study has some limitations. First of all the retrospective nature of the study is intrinsically susceptible to biases. Moreover, different forms of solid tumors were included pre- or post-treatment with various type of therapies as the typology requires at different stages. These variables could ultimately had affected the results.

In our analysis patients were looked independently of treatment and tumor because of the relatively lower number of randomized studies at our disposal. As medicine unfolds more knowledge, a larger number of patients could help to evaluate the impact of our finding and treatment response.

In summary it is known that p53-wt cancers have a better prognosis compared to p53-mut. Our data is not in contradiction with this notion. We observed that serum antibodies generated in the blood of cancer patients against p53 are deleterious. Serological 53 antibodies as biomarker for cancer survival since they can be easily detected with an ELISA method from blood samples, as summarized in a simple workflow in Figure 4, constitute a robust method to be implanted to predict outcome of cancer patients in response to current or future therapies.
Figure 4. Schematic representation of the significance of serological biomarker p53 antibodies (p53Abs) in prediction of cancer survival.

5. Competing interests

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties. No writing assistance was utilized in the production of this manuscript.

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