year cumulative survival rate of 73% against 55% for those aged 55–74 years; patients with residual tumour <2 cm had 78% survival against 50% in those with residual tumour ≥2 cm. These differences, however, were not significant (log rank test, respectively, $P = 0.3$ and 0.1), possibly due to the small numbers of patients. There was no difference in terms of survival according to type of response to first-line chemotherapy.

A study conducted by Copeland and associates in Texas, U.S.A., on 50 patients with microscopic persistent disease found similar results [3]. Patients with only microscopic evidence of disease have a good probability of extended survival, and small residual disease at initial surgery and young age may represent favourable prognostic factors.

1. Richardson GS, Schully RE, Nikrui N, *et al.* Common epithelial cancer of the ovary (part I). *N Engl J Med* 1985, **312**, 415–424.
2. Smith JP, Rutledge F. Chemotherapy in the treatment of cancer of the ovary. *Am J Obstet Gynecol* 1970, **107**, 691–700.
3. Copeland LJ, Gershenson DM, Wharton JT, *et al.* Microscopic disease at second-look laparotomy in advanced ovarian cancer. *Cancer* 1985, 55, 472–478.

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Different Detection Rates of *HER-2/NEU* Overexpression in Ovarian Carcinoma Using Two Different Commercially Available Detection Kits

H. Romero and J. Schneider

ALTHOUGH AN increasing number of reports seem to attribute a prognostic role to *HER-2/NEU* overexpression in human tumours, among which perhaps mammary carcinoma [1, 2] is the most notable, the role of this oncogene is far from being definitively established. A worrying feature when comparing many studies carried out on similar series of tumours, using basically the same detection techniques, is that they often yield contradictory and even totally opposite results. The rate of expression of the *HER-2/NEU* oncogene when using immunohistochemistry for detection particularly seems to vary widely from one group to another. This is especially true when we compare most studies performed on ovarian carcinoma, where variations in results are extreme, with expression rates ranging from 19 to 70% for similar series of tumours [3–5]. Recently, a well conducted methodological study was reported [6], where exactly the same immunohistochemical technique was applied to the tumour samples, for which *HER-2/NEU* amplification had been previously characterised on fresh-frozen aliquots by means of molecular biology techniques. Multitumour blocks were prepared from paraffin-embedded portions of these tumours and cut on to the same glass slides, and immunohistochemistry was carried out only changing the primary antibodies. Twenty-eight antibodies against the c-*erb*-B2 protein (21 monoclonal and seven polyclonal) were tested, and the results could not be more distressing: detection rates varied between 100 and 6%. The conclusion of this study was that the ability to detect *HER-2/NEU* overexpression is extremely variable, and that this could provide an important explanation to the different overexpression rates reported in the literature.

Through a finding that was elicited purely by chance (our laboratory had run out of stock of our usual immunohistochemistry kit, and some samples already processed were erroneously reprocessed using a theoretically identical kit from another manufacturer, with strikingly different results), we were compelled to carry out the present study, for internal quality control purposes.

Twenty-two paraffin-embedded ovarian carcinoma samples were studied by means of immunohistochemistry with the streptavidin–biotin–peroxidase technique, as previously reported by us for fresh-frozen tumour samples [7, 8], after previous deparaffination and rehydration of the slides, using the same monoclonal antibody (NCL-CB11, Novocastra, Newcastle, U.K.), but two commercially available immunohistochemistry kits from different manufacturers (kit A: LSAB streptavidin–biotin–peroxidase immunohistochemistry kit, Dako A/S, Denmark; kit B: Histostain-SP, Zymed, San Francisco, California, U.S.A.). The same positive control was used throughout the study, consisting of paraffin slides from a mammary carcinoma known to overexpress *HER-2/NEU* from a previous study carried out by us on fresh frozen tissue [8]. Although the positive control showed equally positive *HER-2/NEU* detection with either kit, and both with 1-h incubation at room temperature (which is the standard technique recommended by the manufacturer of the antibody), or with overnight incubation at 4°C (which is the technique preferred by us, Figure 1a, b, c), the results for the ovarian carcinoma test specimens were extremely different: 13/22 were clearly positive (59%) with characteristic membrane-bound reaction, using kit A overnight, while only four of 22 (18%) were positive using kit B under the same conditions, and two of 22 (9%) using kit B with the standard technique of 1-h incubation at room temperature (Figure 1d, e, f). It must be stressed once again that the positive control was positive in all three instances, and that the negative controls, carried out in parallel for each of the samples tested by omitting the primary antibody and incubating with blocking serum instead, were negative throughout, thus excluding a non-specific reaction from part of any of the kits.

The conclusions to be drawn from this study are that results of *HER-2/NEU* detection are indeed extremely variable, not only due to differences in the detection rate of different antibodies, such as reported by Press and associates for mammary carcinoma [6], but also using the same antibody, but theoretically equivalent detection kits from different manufacturers, as found here for ovarian carcinoma. In our experience, the range of variation is between 9 and 59%. What is worse is that a positive control from a tumour arising from another (human) organ (a common practice in immunohistochemistry) does not guarantee true negative results if no reaction is detected in the test tissues.

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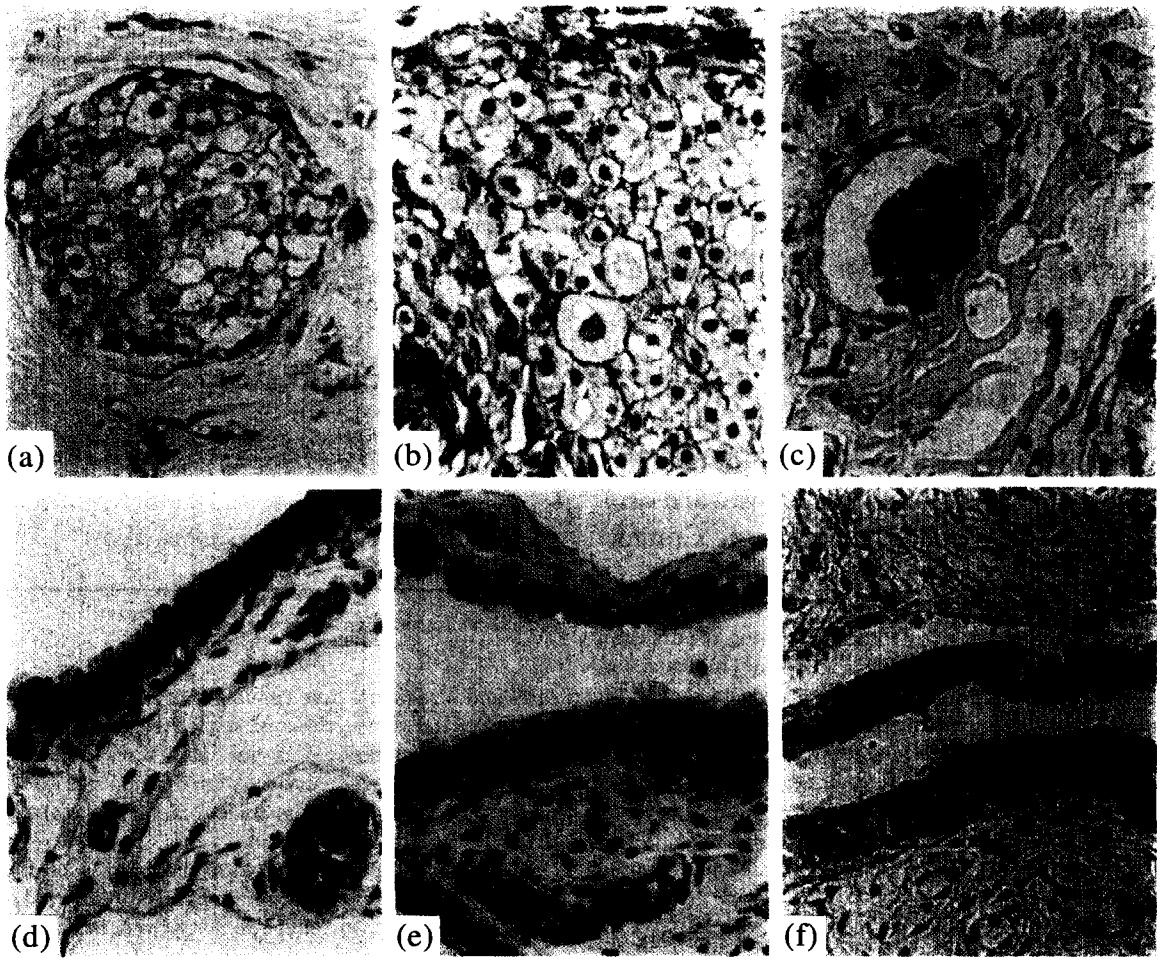

Figure 1. Immunohistochemical detection of *HER-2/NEU* expression in the same specimen of human ovarian carcinoma, using two different detection kits—NCL-CB11 monoclonal antibody, streptavidin–biotin–peroxidase method. (a–c): positive controls of d–f respectively; same mammary carcinoma with known *HER-2/NEU* overexpression. (d) Detection kit B, 1-h incubation at room temperature. (e) Detection kit B, 18-h incubation at 4°C. (f) Detection kit A, 18-h incubation at 4°C.

That the same antigen is consistently detected in some tumours of the same species (mammary carcinoma in our case) using any of the available kits, whereas it shows such variable reactions in tumours from another organ, similarly processed, is something which should alert potential investigators and deserves further study.

1. Gullick WJ, Love SB, Wright C, *et al.* c-*erb*-B2 protein overexpression in breast cancer is a risk factor in patients with involved and uninvolved lymph nodes. *Br J Cancer* 1991, **63**, 434–438.
2. Kallioniemi OP, Holli K, Visakorpi T, Koivula T, Hellin HH, Isola JJ. Association of c-*erb*-B2 oncogene overexpression with high rate of cell proliferation, increased risk for visceral metastases and poor long-term survival in breast cancer. *Int J Cancer* 1991, **49**, 650–655.
3. Meden H, Marx D, Rath W, *et al.* Overexpression of the oncogene c-*erb*-B2 in primary ovarian cancer: evaluation of the prognostic value in a Cox proportional hazards multiple regression. *Int J Gynecol Pathol* 1994, **13**, 45–53.
4. Rubin SC, Finstad CL, Wong GY, Almadrones L, Plante M, Lloyd KO. Prognostic significance of HER-2/neu expression in advanced epithelial ovarian cancer: a multivariate analysis. *Am J Obstet Gynecol* 1993, **168**, 162–169.
5. Hung MC, Zhang X, Yan DH, *et al.* Aberrant expression of the c-*erb*-B2 protooncogene in ovarian cancer. *Cancer Lett* 1992, **61**, 95–103.
6. Press MF, Hung G, Godolphin W, Slamon DJ. Sensitivity of HER-2/neu antibodies in archival tissue samples: potential source of error in immunohistochemical studies of oncogene expression. *Cancer Res* 1994, **54**, 2771–2777.
7. Schneider J, Efferth T, Kaufmann M, *et al.* Expression of the multidrug resistance gene product P-glycoprotein in gynecological tumours. *Cancer J* 1990, **3**, 202–205.
8. Schneider J, Rubio MP, Barbazan MJ, Rodriguez-Escudero FJ, Seizinger BR, Castresana JS. P-glycoprotein, HER-2/neu, and mutant p53 expression in human gynecologic tumors. *J Natl Cancer Inst* 1994, **86**, 850–855.

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