European Journal of Cancer, Vol. 34, No. 13, p. 2135, 1998

Printed in Great Britain
0959-8049/98 $—see front matter

PII: S0959-8049(98)00221-4

Anti-HER-2/neu Antibodies Detected in Sera of Patients with Breast Cancer, but also in Healthy Females

J. Jasinska,[1] C. Wiltschke,[1] T. Brodowicz,[1] C.C. Zielinski,[1] K. Hoffmann-Sommergruber[2] and R. Zeillinger[3]

[1]Clinical Division of Oncology, Department of Medicine I; [2]Department of General and Experimental Pathology; and [3]Department of Gynaecology and Obstetrics, University Hospital, 18–20 Währinger Gürtel, A-1090 Vienna, Austria

RECENTLY, THE presence of anti-HER-2/neu antibodies in 11% of 107 patients with breast cancer, but in none of 200 volunteer blood donors was reported [1]. In our own assays, including an enzyme-linked immunosorbent assay (ELISA) and Western blot, which were designed to detect anti-HER-2/neu antibodies, we arrived at partly contradictory results, which should add a cautionary note to the above-mentioned results.

Sera of 20 patients with early ($n = 10$) or metastatic ($n = 10$) breast cancer and of 20 age-matched healthy control females were analysed for the presence of anti-HER-2/neu antibodies. ELISA was performed by coating plates with 0.5 μg of synthesised c-erbB-2 fragmented peptide coupled with bovine serum albumin (BSA) consisting of 18 amino acids with the sequence PESFDGDPASNTAPLQPC-amid. In order to reduce non-specific binding, a blocking solution (1% milk powder in phosphate-buffered saline (PBS)) was added. A monoclonal mouse antibody directed against the extracellular domain of c-erbB-2 (Genosys Biotechnologies Inc., Cambridge, U.K.) was used for control experiments. Sera derived from patients and controls were diluted 1:50. The plates were incubated overnight at 4°C. A dilution of horseradish peroxidase (HRP)-linked antihuman or antimouse antibody (IgG, final dilution: 1:500; Amersham, Aylesbury, U.K.) was added and incubated at room temperature for 2 h. Enhanced chemiluminescent reagent (Amersham) was used for developing. Chemiluminescence was measured and expressed as relative light units. Assays were performed in duplicate for each serum on wells coated with peptide coupled with maleimide-activated BSA, as well as with BSA only. Values were calculated by defining the difference between counts corresponding to specific binding to coupled peptide and non-specific binding to BSA only. In parallel, Western blot analyses were performed: the membrane fraction of HER-2/neu expressing SK-BR-3 breast cancer cells was separated on a 6% sodium dodecyl sulphate–polyacrylamide gel (SDS–PAGE) and electrotransferred on to nitrocellulose sheets. Strips were processed by saturation in phosphate buffer, pH 7.5, containing 0.5% Tween and 1% milk powder in order to block non-specific binding sites and incubated with diluted (1:50) sera. Bound anti-HER-2/neu IgG was detected by incubation with alkaline phosphatane-conjugated goat antihuman antibody (Jackson Immunoresearch Laboratories, Pennsylvania, Avondale, U.S.A.) and staining with substrate mixture (5-bromo-4-dichloro-3-indolyl phosphate and 4-nitro- blue-tetrazolium chloride, Boehringer Mannheim, Mannheim, Germany). Control assays were performed with rabbit anti-HER-2/neu polyclonal antibodies (Zymed Laboratories Inc., San Francisco, California, U.S.A.) and AP-conjugated swine antirabbit IgG (Dakopatts, Copenhagen, Denmark). To demonstrate the binding specificity of the anti-HER-2/neu antibody, an inhibition assay was performed: serum with high levels of anti-HER-2/neu antibody was incubated with membrane fractions from SK-BR-3 breast cancer cells followed by incubation with nitrocellulose strips. The expected absence of the corresponding band was observed. A detailed description of the employed methods will be given elsewhere.

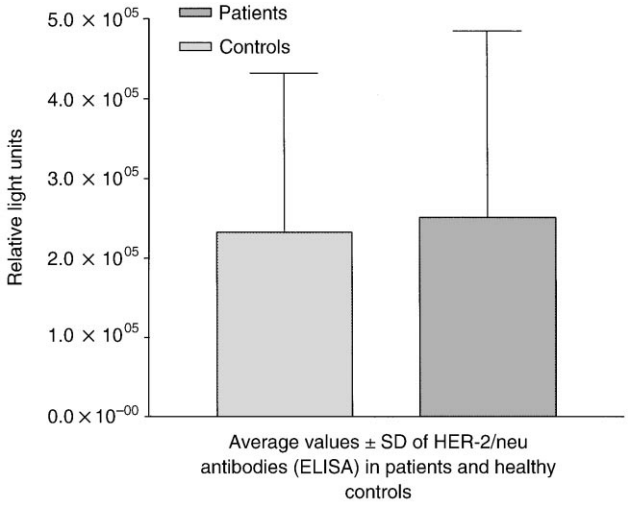

Figure 1. Anti-HER-2/neu antibody levels measured by ELISA.

A close statistical correlation of results obtained in ELISA with Western blot assays ($P = 0.002$; Wilcoxon test) was established. Figure 1 shows the results of ELISA: a wide range of anti-HER-2/neu IgG levels and elevated titres of anti-HER-2/neu antibodies were detected in both groups, patients with breast cancer as well as healthy control individuals. However, no statistically significant difference (Wilcoxon test) in serum levels of anti-HER-2/neu antibodies was found between patients with breast cancer and healthy control females. We thus conclude that the presence of anti-HER-2/neu antibodies might constitute a non-specific phenomenon and may not necessarily correlate with the presence of breast cancer.

Correspondence to C.C. Zielinski.
Received 14 Apr. 1998; accepted 6 May 1998.

1. Disis ML, Pupa SM, Gralow JR, Dittadi R, Menard S, Cheever MA. High-titer HER-2/neu protein-specific antibody can be detected in patients with early-stage breast cancer. *J Clin Oncol* 1997, **15**, 3363–3367.