

Abstract #240

# Normal Levels of Serum EGFr and Decreases in Several Cancers

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### Abstract

Levels of growth factor receptors (such as HER-2/neu) in human tumors have proved to be useful as a guide for monitoring of cancer treatment. Analysis of tumor levels of epidermal growth factor receptor (EGFr) as assayed by immunohistochemistry or tumor lysates has shown a number of cancer types that overexpress the p170 epidermal growth factor receptor (EGFr). These tumors which overexpress EGFr have been targeted for anti-EGFr therapies, several of which are currently in clinical trials. In contrast, serum levels of EGFr have been analyzed much less frequently. We have developed a commercially available sandwich ELISA which measures the 110 kDa extracellular domain (ECD) of EGFr in human serum or plasma samples. Using this assay we have tested over 200 normal sera samples and established a normal mean value of 62 ng/mL with an upper limit of normal of 78 ng/mL and a lower limit of normal of 45 ng/mL. We then analyzed sera samples from a number of cancer patients. Results show a decrease in EGFr ECD levels in cancer patient sera compared to normal controls. Specifically, there were decreases in 42% of the lung cancer sera that were analyzed, in 44% of the late stage prostate, in 48% of the ovarian cancers, in 67% of the colon cancers, in 44% of stage III and 32% of stage IV breast cancers. In addition, upon more detailed analysis of the ovarian cancer sera it was noted that as the stage of ovarian cancer increased, so did the percentage of sera samples which showed decreased EGFr ECD levels. The stage IV ovarian cancers had 78% of the samples which were decreased in EGFr ECD levels below the normal range, while stage I ovarian cancer had 29% and benign ovarian disease sera had 8% of the samples decreased below the normal range for EGFr ECD. Several explanations may help to explain these data. It may be that there is an increase in the amount of EGF, which could then bind the cell-bound EGFr and cause down-regulation of EGFr. Alternatively, there could be the elaboration of anti-EGFr autoantibodies which would bind the EGFr and either take it out of circulation or make it unavailable to the EGFr ECD assay. The determination of serum levels of EGFr ECD may be a useful adjunct to other EGFr measurements for the application of new anti-EGFr therapies.

### Introduction

Growth factor receptors are important in many phases of cell growth and differentiation. The erbB family of growth factors has emerged as critical players in the oncogenic process. Specifically, erb-B1 (EGFr) and erb-B2 (HER-2/neu) are being studied more intensely as molecularly targeted therapies (such as IMC-225 and Herceptin) are becoming available in the fight against cancer.

The majority of EGFr analyses have focused on tissue studies which delineate relative levels and cellular localization of full-length EGFr in tumors and associated normal tissues. A number of cancer types (such as head and neck, ovarian, bladder and colorectal) have exhibited over-expression of EGFr based on tissue levels (1-8). The number of publications which have analyzed EGFr in human serum or plasma samples is rather small in comparison with tissue EGFr analysis. However, there is a growing interest in the measurement of serum EGFr ECD in addition to the traditional tissue-based analysis in light of the anti-EGFr therapies being developed. Among these therapies are small molecule inhibitors of the kinase activity of EGFr (9-12), antisense oligonucleotides (13) and immunotherapies that act directly on EGFr (14-16).

We have used a commercially available microtiter-based EGFr ELISA from Oncogene Science/Bayer Diagnostics to examine serum levels of EGFr extracellular domain (ECD) in normal and cancer patient sera samples.

### Materials and Methods

Human serum samples were diluted in the ELISA kit sample diluent and then analyzed in the Oncogene Science/Bayer Diagnostics EGFr microtiter ELISA. A standard curve with standards tested in duplicate was run in each ELISA. All normal sera samples were tested in duplicate by at least two different operators. Mean values were obtained for all samples tested. A total of 110 normal male and 111 normal female sera were tested in order to determine a normal cutoff. The cutoff was defined as the mean value +/- two standard deviations.

Cancer patient sera samples were then analyzed in the EGFr ELISA, with samples tested in duplicate by at least two different operators. All other experimental parameters were as above.

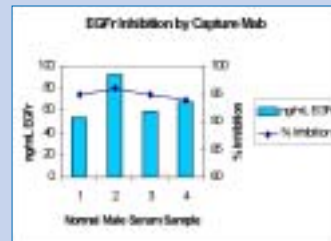


Fig. 1 - Normal Male Serum Inhibition  
Four different normal male sera samples were analyzed for inhibition of assay signal. This shows that the observed assay signal is specific for the analyte of interest. The experiment was carried out by the addition of 10 µg/mL of capture antibody into the sample diluent to block the capture of the analyte by the antibody coated onto the microtiter plate. The signals from the inhibited wells were compared to signals from wells with the same serum sample, but no capture antibody was added as inhibitor. As can be seen in the graph, signals from all four normal male sera samples were inhibited at least 94% compared to uninhibited samples. The average inhibition for all four samples was 95%, thus demonstrating that the signal observed in the normal human sera samples was due to the presence of EGFr ECD.

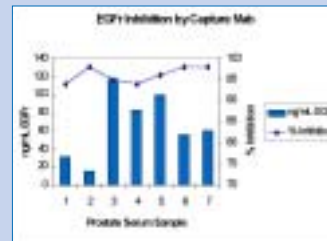


Fig. 2 - Prostate Cancer Serum Inhibition  
Seven different prostate cancer sera samples were analyzed for inhibition of assay signal. This shows that the observed assay signal is specific for the analyte of interest. The experiment was carried out by the addition of 10 µg/mL of capture antibody into the sample diluent to block the capture of the analyte by the antibody coated onto the microtiter plate. The signals from the inhibited wells were compared to signals from wells with the same serum sample, but no capture antibody was added as inhibitor. As can be seen in the graph, signals from all seven prostate cancer sera samples were inhibited at least 94% compared to uninhibited samples. The average inhibition for all seven samples was 95%, thus demonstrating that the signal observed in the prostate cancer sera samples was due to the presence of EGFr ECD.

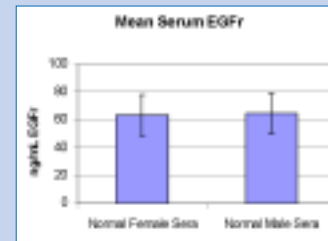


Fig. 3 - Normal Levels of Serum EGFr  
A total of 110 male and 111 female normal sera samples were tested in the EGFr microtiter ELISA. The normal range was calculated by adding or subtracting two standard deviations (SD) to the mean value for each set of samples. For normal male sera the mean was 62 ng/mL (range = 46 - 79 ng/mL) and for normal female sera the mean was 61 ng/mL (range = 45 - 78 ng/mL).

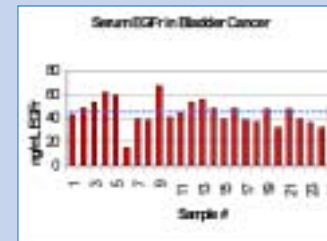


Fig. 4 - Serum EGFr in Bladder Cancer  
A total of 25 bladder cancer plasma samples were analyzed in the EGFr microtiter ELISA. Fourteen of the 25 (56%) showed decreased EGFr levels when compared to the normal range (the lower limit of which is shown as the horizontal line at 45 ng/mL).



Fig. 5 - Serum EGFr in Stage IV Breast Cancer  
A total of 50 stage IV breast cancer sera samples were analyzed in the EGFr microtiter ELISA. Sixteen of the 50 (32%) showed decreased EGFr levels when compared to the normal range (the lower limit of which is shown as the horizontal line at 45 ng/mL).

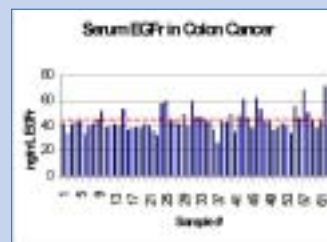


Fig. 6 - Serum EGFr in Colon Cancer  
A total of 50 colon cancer sera samples were analyzed in the EGFr microtiter ELISA. Thirty-three of the 50 (66%) showed decreased EGFr levels when compared to the normal range (the lower limit of which is shown as the horizontal line at 45 ng/mL).

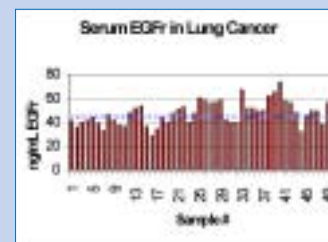


Fig. 7 - Serum EGFr in Lung Cancer  
A total of 50 lung cancer sera samples were analyzed in the EGFr microtiter ELISA. Twenty-one of the 50 (42%) showed decreased EGFr levels when compared to the normal range (the lower limit of which is shown as the horizontal line at 45 ng/mL).

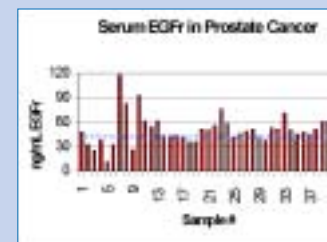


Fig. 8 - Serum EGFr in Prostate Cancer  
A total of 41 late stage prostate cancer sera samples were analyzed in the EGFr microtiter ELISA. Eighteen of the 41 (44%) showed decreased EGFr levels when compared to the normal range (the lower limit of which is shown as the horizontal line at 45 ng/mL).



Fig. 9 - Serum/Plasma EGFr in Ovarian Cancer  
A total of 21 ovarian cancer samples were analyzed in the EGFr microtiter ELISA. Ten of the 21 (48%) showed decreased EGFr levels when compared to the normal range (the lower limit of which is shown as the horizontal line at 45 ng/mL).

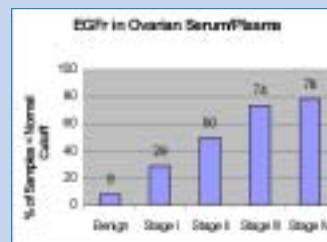


Fig. 10 - EGFr in Ovarian Serum/Plasma  
Various stages of ovarian cancer and benign ovarian disease samples were tested in the EGFr microtiter ELISA. The percentage of samples with decreased EGFr levels (compared to the normal cutoff) increased with increasing stage of ovarian cancer. Specifically, only 29% of the stage I ovarian cancer samples had decreased EGFr serum levels, while 78% of the stage IV ovarian cancer samples had decreased EGFr serum levels.

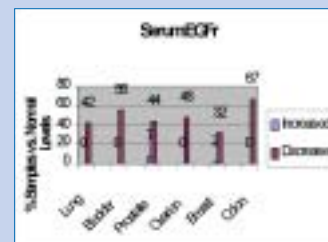


Fig. 11 - Changes in Serum EGFr Levels in Various Cancers  
This graph illustrates that in all the cancer sera samples analyzed in the EGFr microtiter ELISA there was a significant decrease in EGFr levels compared to the normal cutoff (mean - 2 SD). In prostate cancer and stage IV breast cancer there were also a small percentage of samples which showed an increase in serum EGFr levels compared to the normal cutoff (mean + 2 SD).

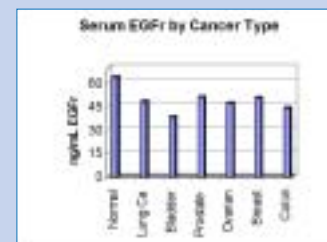


Fig. 12 - Mean Serum EGFr Levels in Various Cancers  
As illustrated in the graph, in all cancer samples analyzed the mean EGFr levels were decreased compared to the mean levels observed in normal sera samples (mean - 2 SD). Analysis of additional serum samples is underway to expand upon these findings.

### Results

The determination of a normal value was defined as the mean value for the 221 normal sera samples +/- two standard deviations. The results from the cancer sera samples were then compared to the normal range. Any of the cancer sera samples that were above the normal range were considered elevated for EGFr and any of the cancer sera samples that were below the normal range were considered decreased for EGFr.

Using over two hundred normal human serum samples, a normal range was established for EGFr ECD levels using the microtiter-based EGFr ELISA. This range was determined by calculating the mean value for normal male and normal female sera samples and adding two standard deviations in order to establish an upper limit of normal EGFr ECD in serum. The range for EGFr ECD in normal male sera was 46 - 79 ng/mL, in normal female sera the range was 45 - 78 ng/mL and for combined males and females the range was 45 - 78 ng/mL.

A variety of cancer sera samples were then analyzed using the EGFr ELISA and results were compared to the normal ranges previously established. Values obtained from the cancer sera showed a decrease in all cancer types examined. Specifically, 42% of the lung cancer sera, 44% of late stage prostate sera, 48% of the ovarian cancer sera, 67% of the colon cancer sera and 46% of the breast cancer sera showed EGFr ECD levels below the normal range. In ovarian cancer there were decreases seen in a larger percentage of the samples as the stage of the cancer increased. Specifically, benign ovarian sera had 8% of the samples below the normal range for EGFr ECD whereas stage I ovarian cancer sera had 29% of the samples decreased, stage II ovarian cancer sera had 50% of the samples decreased, stage III ovarian cancer sera had 44% of the samples decreased and stage IV ovarian cancer sera had 78% of the samples decreased below the normal value for EGFr ECD.

### Conclusions

Using a commercially available microtiter-based ELISA (Oncogene Science/Bayer Diagnostics) for EGFr ECD we determined the range of EGFr ECD in normal male and female sera samples. Utilizing these normal ranges we then examined a number of cancer sera samples. Sera from a variety of cancer patients showed that a large percentage of all types of cancer sera tested showed a significant decrease in EGFr ECD. This ELISA may be useful in conjunction with novel therapeutics directed against the Epidermal Growth Factor receptor.

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