SUMMARY & CONCLUSIONS
Cancer is the second leading cause of death worldwide. 11 million new cases of cancer and 7 million cancer-related deaths were reported in the year 2012. Approximately 25 million people were living with cancer. Despite multimodal intervention strategies initiated to reduce cancer-related mortality, many nations, including the USA and the UK, still grapple with significant cancer mortality rates. To overcome this challenge, the current medical focus has been centered on early cancer detection that enables curative treatment to be administered before cancer progresses to late (most often incurable) stages.

Since many years various proteins and genomic markers have represented the prognostic information of metastatic cancers and helped to predict which patient may benefit most from systemic treatment or targeted therapies. To better understand the progression underlying tumor, the search for potential is highly desirable. Therefore, autoantibodies hold significant promise as tools for early diagnosis and better prognosis of a number of diseases including cancers and autoimmune diseases. Since the immune system is a ready-made and highly sensitive surveillance system, there is expectation that it will sense aberrations caused by diseases and generate autoantibodies as a result well before our current medical technologies can detect them. In order to take advantage of this, we require sufficiently sensitive ways to detect specific autoantibody biomarkers or signatures, which is not an insignificant challenge. It will not only allow for early detection of disease including many cancers, but also a mechanism to track patient’s response to treatment and to improve prognosis. Therefore, we developed a simple technique to detect autoantibodies against HABPs as sensitive common biomarker for early detection of cancer.

- HA-receptors (HABP) were detected using biotinylated HA (bHA) polymer in, healthy control, cancer and other diseases by employing a simple and rapid method ELISA and western blot. The elevated level of HABPs was found in cancer compared to healthy control and other diseases.

- When the blot was reacted with healthy control and breast cancer patients sera that might contain antibodies (autoantibodies) which were reacted with multiple binding proteins based on molecular weight they seems like HABPs.
SUMMARY AND CONCLUSION

• Those tumor associated autoantibodies levels were also varying from cancer to cancer. We speculate that self reactive autoantibodies might be produced to specific binding proteins. These binding proteins (HABPs) might elicit the regulation of autoantibody production in circulating system during tumor development.

• By HA competition experiments the specificity of antibody to HABPs was determined using both ELISA as well as western blot. The results shown the specificity of antibody binding to HA- receptors were inhibited by 89.6% in ELISA (crude sera) where as in western blot completely abolished antibody binding. Therefore, the circulatory antigen (HABPs) might be detected by patients own antibody as self defence mechanism theory.

• To know the specific antibody to HABPs, circulatory autoantibody (IgM) was purified using gel filtration and affinity chromatography in healthy control and breast cancer sera and measured the amount of tumor specific antibody IgM. Their level was down regulated along with tumor progression.

• To know the nature of tumor associated antigen (HABPs), were partially purified by strong anion exchanger chromatography (Q Sepharose). Autoantibody IgM reactive proteins were eluted in 50 and 150mM NaCl fractions. Both fractions were pooled and measured the amount of purified HABPs. Their levels were up regulating along with tumor progression.

• In order to confirm autoantibody IgM reactive proteins might be a HABPs, HA competition experiments were conducted by both ELISA and western blot. In ELISA, HA 91% inhibited IgM binding to its receptors. In western blots moderately abolished IgM binding to HABPs. Therefore, IgM reactive proteins might be a HABP specific.

• IgM reactive proteins homology was determined with standard well characterized HABPs like trance membrane glycoproteins CD44 and cell regulatory proteins Cdc37. By immune pulled down with specific antibodies (as mentioned above
antibodies) and followed by the immune complex was cross reacted with (1) mAbs CD44 and Cdc37 (2) IgM antibody. IgM pulled down proteins were reacted by both CD44 mAbs CD44 and Cdc37 with high affinity. In contrast, mAbs CD44 and Cdc37 pulled down immune complex was also reacted by affinity purified IgM antibody with strong intensity at 80kDa and 50kDa. Therefore, IgM reacting HABPs were mainly involved in many biological functions like cell migration, cell differentiation etc...

- Frequency distribution and mean titer of anti-HABPs autoantibodies were measured in patients with cancers higher frequency 88.6%; mean titer, 0.98), in healthy controls frequency distribution 31.07%; mean titer, 0.25). Above broken line 0.25 mean considered as positive

- The sensitivity and specificity of the anti-HABPs autoantibodies were evaluated using ROC plots, exhibited 91.9% sensitivity and 76.3% specificity (95% confidence interval) in discriminating between patient with cancer and HC and area under curve (AUC) was 0.85. Therefore, Circulating tumor associated anti-HABPs autoantibodies might be used as sensitive common biomarker for detection of multiple malignant tumors.

At present there is little to offer for early diagnosis, even in those at high risk of developing the disease. Autoantibodies have been shown to be present in the circulation of people with various forms of malignancy before cancer-associated antigens can be detected, and these molecules can be measured up to 5 years before symptomatic disease. therefore, detection of autoantibodies to a panel of HA-receptors might have potential to provide clinicians with the opportunity to detect early amplification of the carcinogenic signal, thereby might providing a sensitive, specific and simple screening tool for the early diagnosis and subsequent early clinical intervention of cancers.

In conclusion, elevated level of anti-HABPs autoantibodies detected with greater clinical significant. Characterized both antigen (HABPs) and antibody IgM by ELISA. Mean frequency distribution anti-HABPs autoantibodies in cancer and
healthy controls were determined. Roc plot exhibited greater Sensitivity and specificity of anti-HABPs autoantibodies as a biomarker. The proteins, as detected by an affinity purified IgM their homology was determined with CD44 and Cdc37. It was found that the IgM pulled down antigen was strongly reacted with mAb CD44 and Cdc37. Therefore, these combinations of circulating tumors associated anti-HABPs autoantibodies might be used as a sensitive common biomarker for detection of multiple malignant tumors.