The Clinical Significance of Circulating Tumor Cells (CTC), A Short Literature Review

Today, the importance of tumor cells disseminated into bone marrow as an independent prognostic marker has been widely confirmed. Recently, also the clinical significance of CTC in peripheral blood has been proven.

The latter is of special importance, because the detection of CTC in blood mirrors a dynamic process, whereas the presence of tumor cells in bone marrow might be the result of a process, which became static at some time after onset of the disease. Tumor cells in bone marrow are usually dormant and have a very long survival time **(Karison et al 2000)**, i.e. they are not destroyed be the body's defence mechanisms. Tumor cells can be detected in bone marrow and lymph nodes already at the time of diagnosis of the primary tumor. However, because of their long survival time they are not useful for obtaining a detailed account on the time course of the disease or the success of a therapeutic intervention **(Braun et al 2000)**. In contrast, the survival time of 24 hrs of CTC in blood is relatively short **Patel et al. (2002)**. The accidental detection of these cells seems unlikely unless there is a permanent new afflux of tumor cells. CTC can therefore most likely be detected during tumor progression, which offers besides a prognostic value also independent uses for the evaluation of therapeutic efficacy and for the early recognition of tumor progression. This has been confirmed through numerous clinical studies during the last four years.

CTC as a Prognostic Factor

Stathopoulou et al. (2002) described a significant negative prognosis after detecting CTC by a CK19 RT-PCR method in 128 breast cancer patients (stage I/II). Patients having CTC in blood after removal of the primary tumor and before receiving adjuvant therapy had a significant lower survival time (risk factor 8.5) and a reduced disease free interval (risk factor 5.1). It should be noted that this was a patient group with a relatively favourable prognosis. Therefore, the identification of CTC in these patients

represents an important additional factor for deciding about therapy. This prognostic power in this group was independent of the assessment of the lymph node status, whose prognostic value for disease free survival was clearly inferior (node status >n4 had a risk factor 2.744).

These results were supported by the work of **Xenidis et al. (2003)** and **Giatromanolaki et al. (2004)**, who also could show a significant prognostic value for the estimation of the survival time and disease free interval, when they detected CTC by a CK-19 RT-PCR method. Similar results of somewhat lower significance were obtained by **Gafario et al. (2003)** with an immunocytometric method on 93 patients before surgery and before neo-adjuvant therapy. Patients with cytokeratin positive cells in their circulation (putative CTC) had a shorter survival time as well as a shortened disease free interval, inspite of this method being much less sensitive than a PCR-based analysis (with 1000 tumor cells spiked into 5 ml blood). There was a false negative detection rate of 20-40%).

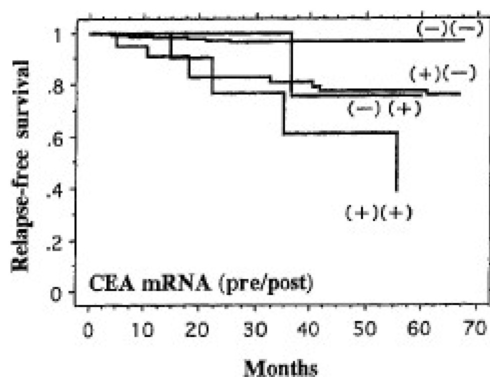

Abb. 1 Jotsuka et al. (2004). Kaplan-Meier analysis showing the correlation between disease free survival and the presence of CTC before and/or after surgery. ((-)(-)= before and after surgery neg.; (+)(+)= before and after surgery pos.; (+)(-) before surg. pos., after surg. neg.; (-)(+) before surg neg, after surg. pos)

A further improvement of the prognostic value was achieved by **Jotsuka et al. (2004)** by investigating 100 n0 breast tumor patients before and after surgical removal of the primary tumor. They determined CEA by a RT-PCR method in a nucleated cell fraction obtained by gradient centrifugation. It could be shown that patients with CTC either before or after surgery had a significant shorter disease free interval than those who had no CTC at any time. Patients, who were CTC positive at both times had the worst prognosis (Fig. 1). Most recently **Cristofanilli, Budd et al. 2004** did show the clinical importance of CTC as an independent prognostic factor in 177 breast cancer patients using the Veridex technology, which led to FDA clearance of the test system

Hardingham et al. (2000) analysed the expression of 4 tumor markers (CK19, CK20, MUC1, MUC2) in blood samples of 94 colorectal cancer patients after a immunomagnetic bead selection of tumor cells employing the BerEP4 antibody. These result also show a clear cut prognostic value for the estimation of the survival time of Dukes A/B patients. These data suggest a chemotherapeutic intervention in patients, who are stratified as Dukes A/B (favourable prognosis) but have CTC. The same conclusion was reached by **Allen-Mersh et al. (2003)** by correlating the presence of CTC 24 hrs after surgery with disease free survival. 70% of relapses could be predicted in a 6 year study with 53 patients. Interestingly, only 1 relapse occurred in 43 patients who were negative with respect to lymph nodes and CTC during the observation period.

A distinct indication for the prognostic significance of CTC is not restricted to breast and colon cancer. **Gewanter et al. (2003)** were able to show an inferior disease progression in patients with tumors of the prostate, who had CTC after radiotherapy. **Shariat et al. (2003)** too could demonstrate, that the detection of PSA mRNA in the blood of tumor patients after surgery correlated with a more aggressive disease progression and an inferior prognosis. **Halabi et al. (2003)** found a significant increase of the risk for a relapse in patients with cancer of the prostate, if CTC could be identified after surgery (risk factor 1.7, CI 95%, p=0.006).

It can be concluded that the detection of CTC can be used to decide about either to commence or to continue an adjuvant therapy or to avoid an unnecessary therapy altogether.

Therapeutic Monitoring

CTC may be an indicator for therapeutic efficacy. During chemotherapy the continuous appearance of CTC in blood would only occur if there was a persistent proliferation process. This may be halted with a successful therapy (stable disease) or might even be reduced (remission). There, the source of CTC and their dissemination would have been removed, which is then associated with the disappearance of CTC from blood.

In immunotherapy of cancer the destruction of the source of the CTC, the CTC themselves and tumor cells in the bone marrow will be the primary target of therapeutic antibodies. The efficacy of this therapy can be immediately assessed through the detection of tumor cells as CTC in blood or in bone marrow aspirates.

Interesting study data are available for both therapeutic interventions confirming these assumptions.

Smith et al. (2000) investigated the presence of CTC with the CK19 RT-PCR method in 22 breast cancer patients during systemic therapy. A correlation of clinical response towards therapy with the detection of CTC was shown in 83%. Inspite the small number of patients one can conclude that the detection of CTC is showing if there is either a therapeutic success or a failure.

Giatromanolaki et al. (2004) has correlated the efficacy of a systemic adjuvant chemotherapy with the presence of CTC in 32 patients (early stages I/II and IIIA). They concluded that the presence of CTC before surgery was predictive for an inferior therapeutic outcome, irrespectively which therapeutic regimen (CMF/FEC or TAC) was used.

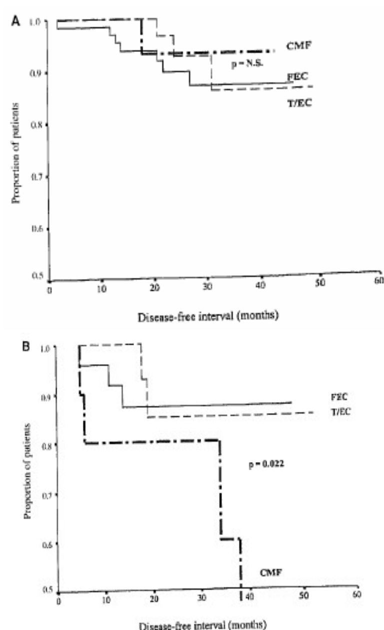

Fig. 2 Xenidis et al (2003). Kaplan-Meier analysis of therapy sucess for patients treated with systemic therapy and without (A) or with CTCs (B). CMF: Cyclophosphamide, Methotrexate, 5-FU; FEC: 5-FU, Epirubicin, Cyclophos-phamide; TAC: Docetaxel, Doxorubicin. Cyclophosphamide

Xenidis et al. (2003) found interesting differences in the response of 161 breast tumor patients towards different systemic therapeutic regimens according to the presence of CTC in blood immediately after cessation of the therapy. A sub-group of patients with a lymph node status <n3 but detectable CTC after therapy had in spite of therapy a 4-fold risk to relapse. Furthermore, there were significant differences depending on the therapeutic regimen being used (Fig. 2). It appears to be possible to obtain information about the individual response to therapies by the detection of CTC in blood and possibly by their further characterization.

Hayes et al. (2002) were able to provide important insights investigating CTC with respect to a possible herceptin therapy by measuring the level of Her2 expression in 20 breast cancer

patients during palliative therapy. In 6 of 8 patients with progressive disease during systemic therapy CTC could be detected also in 3 of 4 patients with stable disease during treatment. However, only 1 of 7 patients responding with complete or partial remission towards the systemic therapy had CTC. Furthermore, in 4 of 20 patients, whose primary tumor was Her2 negative, Her2 positive CTC were found. The detection of newly gained Her2 expression during therapy is implying an inferior prognosis but is also offering a possibility for herceptin therapy which shows the importance of the of CTC analysis in the framework of an individualization of superior therapeutic regimens.

Advantage of AdnaGen's Technology to Detect CTC

There are two reasons for the difficulty to detect CTC in blood:

One reason is the illegitimate transcription of tumor associated mRNA by normal nucleated blood cells (e.g. thrombocytes). As a rule of the thumb it can be stated that about one in a thousand nucleated blood cell produces "illegitimate" tumor associated mRNA. This leads to a strong background, which can vary from person to person, making sometimes the distinction between healthy donors and patients impossible. A lowering of this background level can only be achieved by a selective enrichment of tumor cells or removal of other nucleated cells in blood. Fig. 3 demonstrates that a distinction of the expression patterns between blood of healthy donors and blood spiked with 10 tumor cells cannot be made without a preceding separation step.

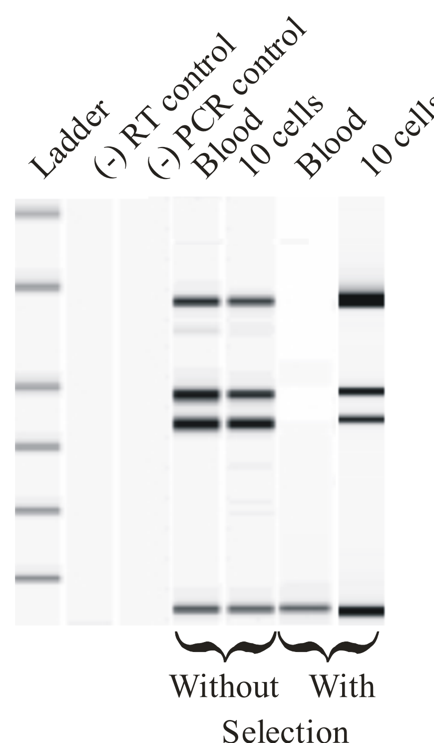

Fig. 4: Preselection of tumor cells is essentially necessary to reach the specificity required for a diagnostic setup. Without selection one cannot distinguish blood samples without tumor cells and samples with spiked tumor cells, whereas this is easily possible if preselection is performed.

Because of the high specificity of immunochemical methods this is done with antibody coated magnetic particles, whereby BerEP4 is a preferred antibody. This antibody recognizes the epithelial antigen EpCAM, which is expressed on most solid tumors.

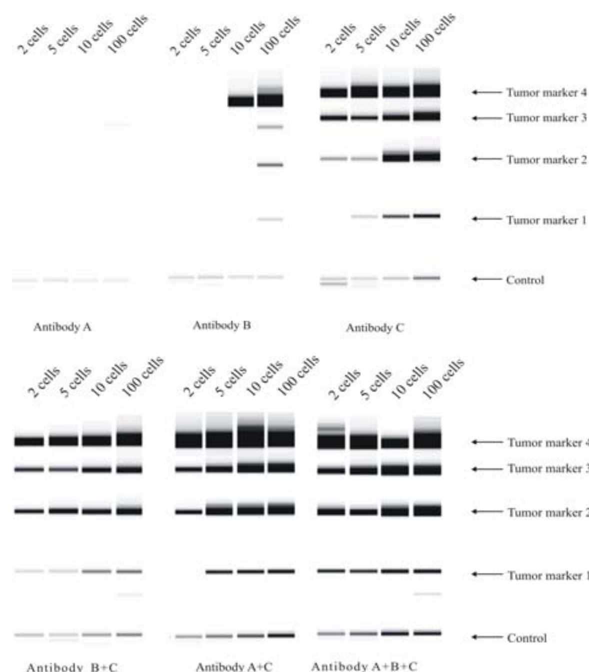

Fig 4: Antibody combinations lead to an synergistic increase in sensitivity.

The other reason is the extremely high variability of surface antigen expression on tumor cells. This variability can lead to a lack of EpCAM expression, thus making these cells elusive for their detection. Only an optimized combination of different antibodies, which are binding to different epitopes of the tumor cells is able to prevent the loss of detection sensitivity of these cells. Fig. 4 clearly shows this effect. The combination of 3 different antibodies for tumor cell enrichment increases the analytical sensitivity profoundly and helps to avoid false negative results. By this procedure the detection of CTC by analysis of tumor associated mRNA yielding high analytical sensitivity whilst maintaining a high specificity becomes possible. It is essential that the variability of tumor cell expression is also taken care of in the succeeding RT-PCR reaction. In spite of the high analytical sensitivity of the PCR reaction the determination of just a single marker (e.g. CK19) may lower the clinical sensitivity, because the chosen tumor marker is not expressed in all cases. This leads to unpredictable variable expression patterns in patients' tumor cells which may yield false negative results if this fact is not taken care of. Variations during therapy and at different time points of sample taking in a single patient have also been observed.

The AdnaTests take care of this problem by using a further combination of tumor markers on mRNA level. With this modification the clinical sensitivity could decisively be improved.

Only by combining a highly specific immunomagnetic cell selection system using an optimized antibody combination with the highly sensitive RT-PCR technology using a

combination of the mRNA tumor markers ("Combination of Combinations Principle") the required clinical specificity and sensitivity for a valid diagnostic application could be assured.

Conclusion

After some controversial findings in the beginning of the research on CTC it could be clearly shown in recent years, that the detection of CTC in the blood of tumor patients is a valid new parameter to monitor disease progression and to optimize therapy. In spite of this also the recent publications have to be taken with a pinch of caution, since lack of sensitivity (immunocytometry) or specificity (RT-PCR without cell selection) cannot be ruled out, which complicates the transfer of statistical data to individual patient situations. Through the combination of independent test systems into one AdnaGen succeeded to almost exclude possible sources of false results and having done so is enabling the valid diagnostic use of CTC detection in the blood of tumor patients.

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