

PDF hosted at the Radboud Repository of the Radboud University Nijmegen

The following full text is a publisher's version.

For additional information about this publication click this link.

<http://hdl.handle.net/2066/25601>

Please be advised that this information was generated on 2017-12-05 and may be subject to change.

Detection of tumour DNA in serum of colorectal cancer patients

J. B. DE KOK,* W. W. VAN SOLINGE,*† T. J. M. RUERS,‡
R. W. H. M. ROELOFS,* G. N. P. VAN MUIJEN,§ J. L. WILLEMS* &
D. W. SWINKELS*

Departments of *Clinical Chemistry, ‡Surgery, and §Pathology, University Hospital Nijmegen, Nijmegen, †Eemland Hospital, Department of Clinical Chemistry, Amersfoort, The Netherlands

de Kok JB, van Solinge WW, Ruers TJM, Roelofs RWHM, van Muijen GNP, Willems JL, Swinkels DW. Detection of tumour DNA in serum of colorectal cancer patients. *Scand J Clin Lab Invest* 1997; 57: 601-604.

Circulating tumour DNA has previously been detected in serum and plasma of patients with lung cancer and head and neck cancer. These observations could potentially lead to new, specific and non-invasive tools for diagnosis, prognosis and follow-up in neoplastic disease, if found to be a more general phenomenon. To test if tumour DNA is also present in serum of patients with colorectal cancer, we selected 14 colorectal cancer patients with advanced disease. In seven patients, K-ras mutations were detected in the primary tumour, using mutant-specific primers for point mutations in codon 12 or 13 of the K-ras gene. All patients were analysed for mutant DNA in serum. Tumour-specific point mutations, corresponding to the K-ras mutations found in the primary tumour were detected in the serum of all patients but one. No mutant K-ras could be detected in the serum of seven patients without K-ras mutations in the primary tumour. These results may be useful in assessing tumour burden in patients with neoplastic disease. Moreover, consecutive testing of serum tumour DNA after surgery or chemotherapy may be used as a tumour marker for recurrent disease.

Key words: circulating DNA; K-ras; mutation screening

D. W. Swinkels, University Hospital Nijmegen St Radboud, Department of Clinical Chemistry, Geert Grooteplein 8, 6500 HB Nijmegen, The Netherlands

Detection of free circulating tumour DNA in the serum of cancer patients offers new possibilities for cancer diagnostics. Recently, sensitive polymerase chain reaction (PCR)-based assays have been used to detect mutated tumour DNA sequences in the serum or plasma of patients with lung cancer and head and neck cancer [1, 2]. Circulating tumour DNA was predominantly found in patients with advanced disease and in those with poor prognosis. It was suggested that analysis for circulating tumour

DNA might be useful in tumour staging and might help to identify patients at risk of tumour recurrence [3]. These findings are provocative and call for further investigation in other types of cancer.

Mutations in codon 12 or 13 of the K-ras gene occur in approximately half of all colorectal cancers. The detection of these mutations against a background of normal DNA requires highly sensitive and properly validated assays. Mutant allele-specific amplification (MASA) by PCR uti-

lizes the inability of Taq polymerase to elongate a primer when the 3' end of the primer mismatches the template and can therefore be used to selectively amplify mutated sequences [4].

In this study, patients with advanced colorectal cancer were evaluated for circulating mutant tumour DNA in their serum. Analyses were based on the detection of point mutations of the K-ras gene in serum by MASA.

MATERIALS AND METHODS

Fourteen patients with advanced colorectal cancer (Dukes D) were selected.

Six 8 µm, formalin-fixed, paraffin-embedded tissue sections of the colorectal primary were placed in an Eppendorf tube. Six hundred microlitres of cell lysis solution (Puregene, Gentra Systems, Research Triangle Park, NC, USA) was added and heated for 10 min at 80°C in a thermomixer (Eppendorf, Hamburg, Germany). After centrifugation for 1 min at 23 900×g, 10 µl proteinase K (20 mg/ml) (Boehringer Mannheim GmbH, Mannheim, Germany) was added and protein digestion was performed overnight at 55°C in a thermomixer. Samples were further processed according to the manufacturers protocol for DNA isolation from tissue (Puregene). DNA content was quantified spectrophotometrically and 5 µl, containing 300 ng DNA, was used for MASA.

Blood was collected in Corvac tubes (Sherwood Medical, Ballymoney, N. Ireland). After clotting, tubes were centrifuged for 10 min at 2000×g and serum was stored at -80°C until use.

DNA isolation from serum samples was performed using a modified protocol for DNA isolation from body fluids according to the Puregene instructions. Briefly, 500 µl serum was added to a 1.5 ml Eppendorf tube containing 500 µl cell lysis solution (Puregene), 10 µg poly[A] DNA (Sigma, Zwijndrecht, The Netherlands) and 15 µl proteinase K (20 mg/ml). After protein digestion overnight at 55°C in a thermomixer, samples were cooled on ice. Two hundred microlitres of protein precipitation solution was added and the samples were incubated on ice for 15 min after mixing. Proteins were pelleted by centrifugation for 3 min at 23 900×g. DNA was precipitated with isopro-

panol, washed once with 70% ethanol and dissolved in 50 µl 10 mM Tris-HCl (pH 8.5). Five microlitres was used for MASA.

MASA was performed using 20 pmol of each primer, 50 µM of each dNTP, 2.5 mM MgCl₂, 1 U AmpliTaq Gold (Perkin Elmer Corporation, Foster City, CA, USA), 5 µl 10×PCR II buffer (Perkin Elmer) and 5 µl sample in a total volume of 50 µl. An initial sample denaturation and polymerase activation step at 94°C for 10 min was followed by 35 cycles of 94°C for 1 min, 60°C for 30 s, 72°C for 45 s and a final extension of 10 min at 72°C. MASA forward primers for mutant K-ras amplification were identical to those used by Hasegawa *et al.* [4]. Primer #103, 5'-ACTCATGAAAATGGTCA-GAGAAACCTTTAT was used as antisense primer. PCR products (178 bp) were separated on a 2% agarose gel (Boehringer Mannheim GmbH) in 0.5×TAE buffer.

RESULTS

Serum and tumour tissue of 14 patients with colorectal cancer were analysed for point mutations in codons 12 and 13 of the K-ras gene. All patients had systemic disease. Seven patients were positive for K-ras mutations in the primary tumour. For all positive patients but one, point mutations in the K-ras gene were present in the serum (Fig. 1), identical to the mutations detected in the corresponding primary tumour. Patients with no K-ras mutation in the tumour were negative for mutant K-ras in serum. To assess the specificity of the method, all MASA primers (four sets of three primers matching the first two nucleotides of codons 12 and 13) were tested on serum of these patients (data not shown). The method is highly specific for mutant K-ras and no false positive signals due to breakthrough of mutant-specific primers have been detected.

DISCUSSION

In this study we demonstrate the presence of tumour-specific DNA in the serum of patients with advanced colorectal cancer. These results, in a group of patients with a common type of

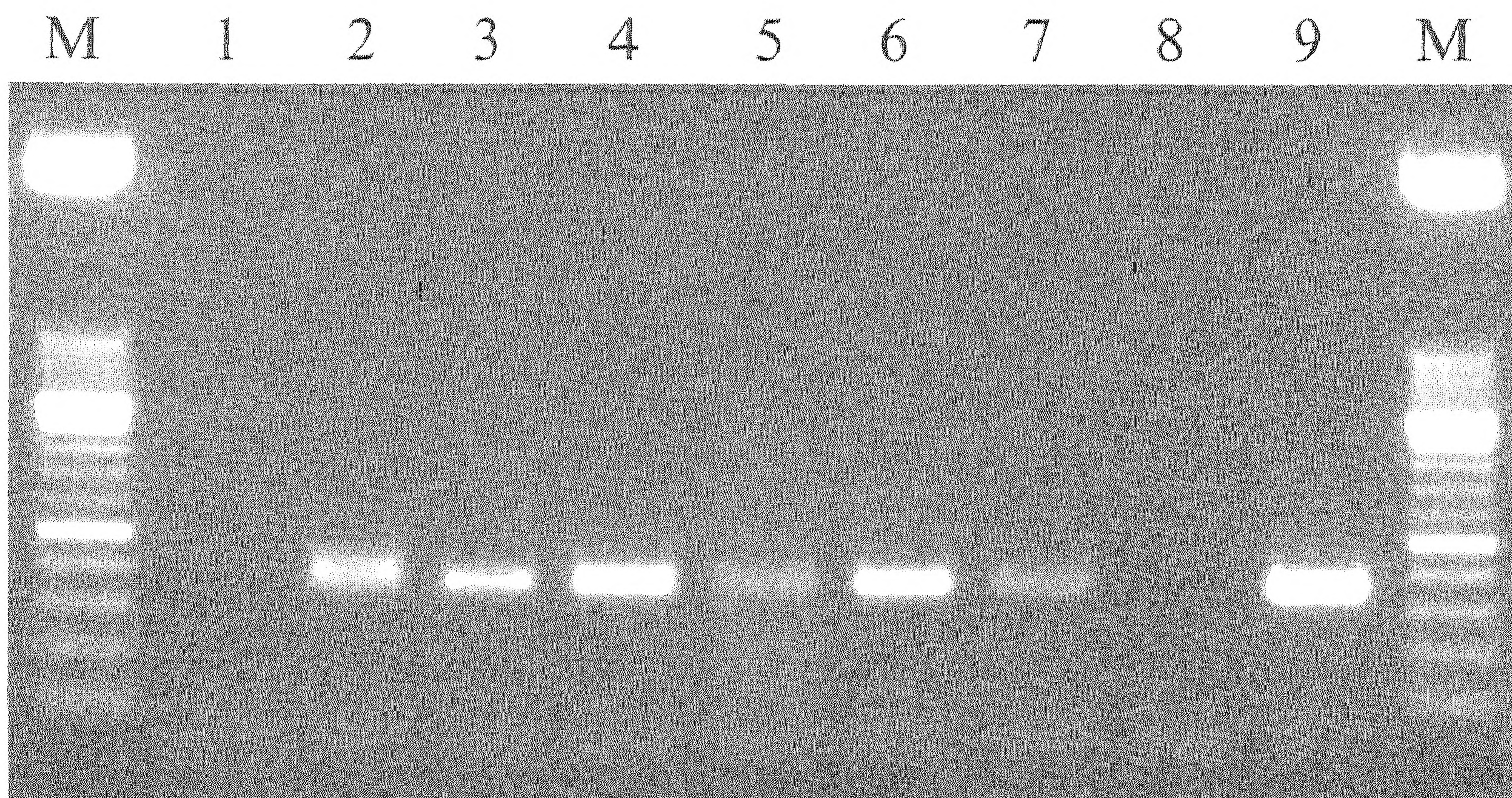


FIG. 1. PCR amplification of mutant K-ras DNA isolated from serum of colorectal cancer patients. Lanes M, 50 bp DNA ladder. Lanes 1–7, mutant allele specific amplification on DNA isolated from serum of 7 colorectal cancer patients with K-ras mutations in the primary tumour. Lane 8, no template control. Lane 9, 100 ng DNA from SW480, a mutated K-ras cell line (positive control).

cancer, consolidate previously published data [1, 2, 5].

Despite the high sensitivity of the MASA, in one patient no K-ras mutation could be detected in the serum, although the primary tumour in this patient was positive for mutated K-ras. It has been reported that metastatic lesions may be genetically different compared to the primary tumour they originate from, due to the presence of heterogeneous clones [6, 7]. This could possibly explain the findings in this particular patient, indicating that the use of more than one tumour marker may be necessary to obtain higher sensitivity.

Analysis of free plasma tumour DNA could potentially be an attractive diagnostic and prognostic tool in patients with colorectal cancer. Recently, Anker *et al.* reported the presence of K-ras mutations not only in the plasma of patients with Dukes D, but also in three patients with Dukes A, B and C stages of colorectal cancer, using a nested PCR technique [5]. These results suggest that circulating tumour DNA sequences may be used for early detection of colorectal neoplasms. If free serum or plasma tumour DNA proves to be a reliable indicator of systemic disease (instead of early disease) at the time of surgery for the colorectal primary tumour, it would enable clinicians to identify patients with poor prognosis who are at risk for tumour recurrence after surgery. This would improve staging and hence selection of those

patients who would benefit most from adjuvant chemotherapy. Moreover, consecutive testing for the presence of free serum tumour DNA in the follow-up after surgery could be used as a tumour marker for recurrent disease. Although our results are preliminary, they do validate efforts to further investigate the potential usefulness of tumour DNA in serum in the diagnosis, staging and follow-up of patients with colorectal cancer.

ACKNOWLEDGMENT

We thank Prof. Dr T. Wobbes (University Hospital Nijmegen, Department of Surgery) for the collection of serum samples

REFERENCES

- 1 Chen XQ, Stroun M, Magnenat JL, Nicod LP, Kurt AM, Lyautey J, *et al.* Microsatellite alterations in plasma DNA of small cell lung cancer patients. *Nat Med* 1996; 2: 1033–4.
- 2 Nawroz H, Koch W, Anker P, Stroun M, Sidransky D. Microsatellite alterations in serum DNA of head and neck cancer patients. *Nat Med* 1996; 2: 1035–7.
- 3 Mulcahy HE, Croke DT, Farthing MJG. Cancer and mutant DNA in blood plasma. *Lancet* 1996; 348: 628.
- 4 Hasegawa Y, Takeda S, Ichii S, Koizumi K,

- Maruyama M, Fujii A, *et al.* Detection of K-ras mutations in DNAs isolated from feces of patients with colorectal tumors by mutant-allele-specific amplification (MASA). *Oncogene* 1995; 10: 1441–5.
- 5 Anker P, Lefort F, Vashioukhin V, Lyautey J, Lederrey C, Chen XQ, *et al.* K-ras mutations are found in DNA extracted from the plasma of patients with colorectal cancer. *Gastroenterology* 1997; 112: 1114–20.
- 6 Oudejans JJ, Slebos RJC, Zoetmulder FAN, Mooi WJ, Rodenhuis S. Differential activation of ras genes by point mutation in human colon cancer with metastases to either lung or liver. *Int J Cancer* 1991; 49: 875–9.
- 7 Nagel S, Borisch B, Thein SL, Oestreicher M, Nöthiger F, Birrer S, *et al.* Somatic mutations detected by mini- and microsatellite DNA markers reveal clonal intratumor heterogeneity in gastrointestinal cancers. *Cancer Res* 1995; 55: 2866–70.

Received: 4 July 1997

Accepted: 5 August 1997