Appendix A: Summary of evidence from surveillance

Exceptional surveillance review 2017 – Metastatic malignant disease of unknown primary origin in adults (2010) NICE guideline CG104

Summary of evidence from surveillance

Diagnosis

Q – 01 For patients with malignancy of undefined primary origin, does immuno-histochemical analysis result in improved outcomes?

Recommendations derived from this review question

Immunohistochemistry

1.2.2.7 Use a panel of antibodies comprising cytokeratin 7 (CK7), CK20, thyroid transcription factor-1 (TTF-1), placental alkaline phosphatase (PLAP), oestrogen receptor (ER; women only) and PSA (men only) in all patients with adenocarcinoma of unknown origin.

1.2.2.8 Use additional immunohistochemistry to refine the differential diagnosis, guided by the results of the panel of antibodies in recommendation 1.2.2.7 and the clinical picture.

Gene-expression-based profiling

1.2.2.9 Do not use gene-expression-based profiling to identify primary tumours in patients with provisional CUP.

Surveillance decision

This review question should not be updated.

Immunohistochemical testing

2017 exceptional surveillance summary

Multiple tumour markers

A study[1] assessed CK7 and CK20 immunophenotype and caudal type homeobox 2 (CDX2) expression in differentiating colorectal, pancreatic and gastric adenocarcinomas. Overall, samples from 118 colorectal adenocarcinomas, 59 gastric adenocarcinomas, and 32 adenocarcinomas were tested.

- CDX2 was expressed in 97% of colorectal, 61% of gastric, and 16% of pancreatic adenocarcinomas. CDX2 expression at 2 cut-off levels (>5% and >50%) had a higher sensitivity (96.6% and 78%) than the CK phenotype.
- CK7−/CK20+ expression was seen in 64% of colorectal 5% gastric, and 0% of pancreatic adenocarcinomas. The specificity for predicting colorectal carcinoma was 96.7%. The authors noted that this was superior to that of CDX2 expression.
- CK7+/CK20+ expression was seen in 20% of colon, 48% of gastric and 22% of pancreatic adenocarcinomas.
- CK7+/CK20− expression was seen in 2% of colorectal adenocarcinomas.

A study[2] assessed the expression of GATA binding protein 3 (GATA-3) and forkhead box A1 (FOXA1) in ER- positive, HER2-positive, and triple-negative breast carcinomas as well as in hepatocellular, colonic, pancreatic, gastric, endometrial, lung, prostatic, renal cell, urothelial, and ovarian serous carcinomas, and

in primary and metastatic melanomas and mesotheliomas. However, the number of samples analysed was not reported in the abstract.

- Oestrogen-receptor positive breast carcinomas showed GATA-3 staining in 96.6% and FOXA1 staining in 96.2%.
- Triple-negative breast carcinomas, showed GATA-3 staining in 21.6% and FOXA1 staining in 15.9%.
- GATA-3 staining was seen in 70.9% of urothelial carcinoma.
- FOXA1 staining was seen in 87.5% of prostatic and 5.1% of urothelial carcinomas, and 40.0% of mesotheliomas.

A study[3] assessed Notch 1, 2, and 3, Jagged 1, MET proto-oncogene receptor tyrosine kinase (cMET), and phosphorylated mitogen-activated protein kinase (pMAPK) expression in 100 samples of CUP. Notch3 was expressed in 97% of CUP samples. Notch3 and cMET expression showed significant linear correlation and were significantly more common in squamous cell carcinomas (positive in 90% of samples). cMET was associated with the longest overall survival. pMAPK was expressed in 91% of CUP samples. It was associated with shortest overall survival.

A study[4] assessed MOC-31 immunostaining in 215 'effusion specimens and washings'. Malignancy was detected in 44% of the samples. MOC-31 was positive in 87% of adenocarcinomas. The adenocarcinomas negative for MOC-31 included lung (n=4), stomach (n=2), colon, breast (n=2), and renal (n=1). The sensitivity of MOC-31 for metastatic adenocarcinoma was 89%; the specificity was 100%; the negative predictive value was 92%; and the positive predictive value was 100%. Mesothelial cells and mesothelioma showed minimal focal cytoplasmic staining in 13% of cases.

A study[5] assessed expression of annexin A10 (ANXA10) as a diagnostic marker in 1,327 samples of primary carcinomas of major organs. Normal ANXA10 expression was seen in the gastric mucosa, the Brunner gland of the duodenum and the urothelium, but expression was absent in other normal gastric tissue. ANXA10 expression was seen in 46% of gastric, 72% of ampullary, 78% of pancreatic and 33% of biliary adenocarcinomas. ANXA10 was expressed in 83% of non-invasive urothelial carcinomas, but was expressed in only 9% of invasive urothelial carcinomas. ANAX10 was rarely expressed in carcinomas of other organs. Of 227 metastatic adenocarcinomas, ANXA10 was expressed in 83% of metastatic pancreatic and 47% of metastatic gastric adenocarcinomas, but was expressed in only 2% of metastatic adenocarcinomas from other organs. In liver metastases, ANXA10 had sensitivity of 83% and specificity of 95% for identifying the pancreas as the primary site of metastatic adenocarcinoma.

A study[6] assessed expression of keratin sulfate (KS) in 102 samples of normal epithelia and 110 samples of carcinomas from the female genital, digestive organs, urinary tract, lung, mammary gland, thyroid and mesothelium.

- In normal tissue, KS was detected in most samples from the female genital tract, but was also detected in samples from the lung, mammary gland, and thyroid. KS was expressed rarely or not at all in the digestive organs and urinary tract.
- In malignant tissue, KS was detected in most samples from endometrial, ovarian and fallopian tube carcinomas, but was also detected in lung, mammary gland, thyroid, pancreatic, and mesothelium carcinomas. KS was not detected in any gastrointestinal, liver, or urinary tract carcinomas.
- Among carcinomas of the female genital tract, digestive organs and urinary tract, KS positivity suggested the possibility of female genital tract carcinomas, with sensitivity of 79.5% and specificity of 92.9%.

A study[7] assessed the staining quality of the monoclonal antibodies 8F1 and D-10 against excision repair cross-complementation group 1 (ERCC1) in 117 samples of malignant cells obtained from pleural (n=75) and peritoneal effusion (n=42) and 10 samples each of lung, breast, and ovarian carcinoma. Immunoreactivity was semi-quantitatively scored for intensity and intensity multiplied by percentage staining (H-score). Tumours were classified as: 39 female genital tract carcinomas; 23 lung adenocarcinomas; 15 mesotheliomas; 14 unknown primaries; 10 breast carcinoma; 12 gastrointestinal carcinomas and 4 other cancers. Reproducible nuclear ERCC1 immunoreactivity was achieved with both antibodies, although D-10 was slightly weaker with more background staining and more variation at low expression levels. Lung

and breast carcinomas had significantly lower expression than other tumour types with 8F1 staining.

A retrospective study[8] assessed 74 CUP samples positive for CDX2. The predominant sites of metastases included liver, carcinomatosis, and nodes; 20 patients had ascites on presentation. Based on immunohistochemistry, 2 cohorts were created: CDX2+, CK20+, CK7− 'consistent with lower gastrointestinal profile' (n=32) with median survival of 37 months; and CDX2+, irrespective of CK7/CK20 status 'probable lower gastrointestinal profile (n=36) with median survival of 21 months. The authors noted that these results indicated that site-specific therapy was associated with improved survival in CUP showing markers of lower gastrointestinal cancer. On multivariate Cox regression analysis, only liver metastases were found to negatively influence survival.

A study[9] assessed an immunohistochemistry algorithm that used 15 tumour markers to classify CUP by predicted site of origin in 71 samples from people with adenocarcinoma of unknown origin. The algorithm had sensitivity of 80.3% and specificity of 97.6%. CUP with a gynaecological profile had the highest response rate to chemotherapy, with significantly longer median progression-free survival compared with other CUP profiles. CUP with lung profile also had significantly longer median progression-free survival than other predicted sites of origin.

Gastrointestinal cancer markers

A study[10] assessed expression of the special AT-rich sequence-binding protein 2 (SATB2) in 840 cases of primary and metastatic colorectal cancer in which immunohistochemistry testing for CK20 was deemed to be necessary for a final diagnosis. SATB2 showed sensitivity of 93% and specificity of 77% to determine a cancer of colorectal origin and in combination with CK7 and CK20 results, the specificity increased to 100%.

A study[11] assessed expression of SATB2 in 9 independent cohorts with primary and metastatic colorectal carcinoma totalling 1,882 samples. SATB2 was positive in 85% of all colorectal cancers, and 97% of colorectal cancers were positive for SATB2 or CK20 or both.

A retrospective study[12] assessed expression of TTF-1 in 91 samples of gastric adenocarcinoma. Up to 25% of samples tested with the antibody clone SPT24 were positive for TTF-1 some of these samples were also positive for Napsin A. However, TTF-1 was not detected in most cases tested with the 8G7G3/1 antibody clone.

A study[13] assessed cadherin-17 and SATB2 expression in 18 samples of medullary carcinoma of the large intestine, 1,941 other tumour samples, and 358 normal tissue samples. The medullary carcinoma samples were additionally tested with a range of other immunomarkers.

- Cadherin-17 and SATB2 were not expressed in 89% of medullary carcinomas.
- Cadherin-17 was expressed in 97% of colorectal adenocarcinomas and in 3.6% of non-gastrointestinal tumours.
- SATB2 was expressed in 98% of colorectal adenocarcinomas and 3.3% of non-gastrointestinal tumours.
- MutL homolog 1 (MLH1) and PMS 1 homolog 2 (PMS2) were not expressed in more than 80% of medullary carcinomas. In medullary carcinoma, focal expression was seen for trefoil factor 3 (TFF3; 72% of cases), mucin 4, cell surface associated (MUC4; 72%), calretinin (67%), CDX2 (67%), CK20 (28%), and synaptophysin (17%).

Breast cancer markers

A retrospective study[14] assessed the expression of GATA-3 (using 2 clones of the GATA-3 antibody, L50-823 and HG3-31), GCDFP-15, and mammaglobulin A in in 338 samples of carcinoma. It was not clear from the abstract exactly what types of carcinoma were studied, or how many samples of each type of cancer were tested.

- GATS-3 was positive in 96% of non-triple-negative breast cancers (for both antibody clones). In triple-negative breast cancers, the L50-823 clone was positive in 87%, and the HG3-31 clone was positive in 63%. The L50-823 clone had lower specificity for identifying breast carcinomas (84%) than the HG3-31 clone (97%).
- GCDFP-15 was expressed in 69% of non-triple-negative breast cancers and 10% of triple-negative breast cancers.
- Mammaglobin A was expressed in 61% of non-triple-negative breast cancers and 17% of triple-negative breast cancers.

A study[15] evaluated a panel of breast-specific markers and markers that are expressed in tumours resembling breast cancer in a single data set of breast cancers. The exact number of samples tested was not clear in the abstract, but appeared to be around 198.

- GATA-3 was positive in 95%.
- CK7 was positive in 92%.
- Androgen receptor was positive in 80%.
- Oestrogen receptor was positive in 80%.
- Progesterone receptor was positive in 69%.
- NY-BR-1 was positive in 55%.
- Mammaglobin was positive in 52%.
- Vimentin was positive in 31%.
- Gross cystic disease fluid protein-15 (GCDFP-15) was positive in 26%.
- CK20 was positive in 0.5%.
- Paired box transcription factor 8 (PAX8) was positive in 0%.

Although most breast carcinomas were CK7+/CK20−, a CK7−/CK20− profile was seen in about 8% of cases.

A study[16] assessed 29 metastatic breast tumour samples that tested under the assumption that they were CUP and 48 samples of other tissue assumed to be negative controls.

- Mammaglobin was positive in 34% of breast cancers and 4% of control tissues.
- GCDFP-15 was positive in 45% of breast cancers and 8% of control tissues.
- Oestrogen receptor was positive in 69% of breast cancers.
- CK7 was positive in 86% of breast cancers.

The diagnostic strategy with the highest positive predictive value (88%) included hormone receptors and mammaglobin in series.

Genitourinary cancer markers

A retrospective study[17] assessed the expression of TTF-1 in 62 samples of primary ovarian carcinoma and 15 samples of normal ovarian tissue using 2 clones of the TTF-1 antibody (SPT24 and 8G7G3/1). Nuclear expression of the SPT24 clone of TTF-1 was detected in 17.7% ovarian carcinomas and expression of the 8G7G3/1 clone of TTF-1 was detected in 3.2% of ovarian carcinomas, respectively. Positive cytoplasmic immunostaining of clone SPT24 was detected in 1.6% of cases. In contrast, normal ovarian tissue showed negative expression of both clones. A significant inverse relationship was observed between expression of the TTF-1 SPT24 clone and tumour stage, whereby expression may indicate good prognosis.

A study[18] assessed expression of the oncofetal antigen glypican 3 (GPC3), PAX8, and the DELTAN isoform of p63 (p40), in 107 samples of squamous cell carcinomas from 11 anatomic sites and 49 urothelial carcinomas.

- GPC3 was detected in 20% of squamous cell carcinomas and 12% of urothelial carcinomas.
- PAX8 was detected in 3% of squamous cell carcinomas limited to the uterine cervix and 10% of urothelial carcinomas.
- p40 was detected in 99% of squamous cell carcinomas and 96% of urothelial carcinomas.

Hepatic and biliary markers

A retrospective study[19] assessed expression of TTF-1 and Napsin-A (NapA) in 33 samples of liver, gallbladder, and pancreato-biliary tissue assumed to be cholangiocarcinoma and 26 samples of non-neoplastic intrahepatic and extrahepatic biliary epithelium tissue as controls. Immunohistochemical analysis for was performed and graded for intensity and quantity. TTF-1 was negative in control biliary tissue but positive in 27.2% of cholangiocarcinomas. All 9 TTF-1-positive cases were extrahepatic, and most arose from the upper biliary tract (gallbladder and hepatic ducts). TTF-1 positivity was associated with age 60 years and older, but not with sex. Three TTF-1-positive cases were also NapA positive.

A study[20] assessed 2 rabbit antibodies (monoclonal and polyclonal) against arginase (ARG-1) in 195 samples of hepatocellular carcinoma, and 5,704 samples from other cancers. The monoclonal antibody against ARG-1 stained 82% of hepatocellular carcinomas and the polyclonal antibody against ARG-1 stained 81%. The monoclonal antibody against ARG-1 was negative in all other cancers except in 3 of 64 prostate adenocarcinomas and in 1 of 12 cholangiocarcinomas.

Topic expert feedback

Topic expert feedback suggested that OCT3/4 (also known as POU class 5 homeobox 1) was

a better target for immunohistochemistry to identify germ line cancers testing than PLAP, which is recommended in the guideline.

Impact statement

The evidence suggests that lack of specificity remains a challenge for immunohistochemical testing, and sensitivity can also be imperfect. Furthermore, detection may differ depending on the antibody clone used.

In many studies, the number of samples tested was small and there is an absence of any new systematic review of the evidence in this area. None of the new evidence allowed evaluation of the topic expert feedback that OCT3/4 is a better target than PLAP.

There is some evidence[9] that an algorithm may allow tumour classification with greater specificity, with indications of improved patient outcomes with site-specific therapy. The set of initial immunohistochemistry tests recommended in the guideline were chosen to achieve a similar result.

There was no clear evidence that any of the targets assessed in the studies identified in surveillance should be added to the panel of immunohistochemistry tests that are currently recommended. These other markers may be useful for additional immunohistochemistry to refine the differential diagnosis, as is currently recommended.

New evidence is unlikely to change guideline recommendations.

Tumours markers in pleural effusion

A study[21] assessed levels of carcinoembryonic antigen (CEA), alpha fetoprotein (AFP), CA125, CA153 and CA199 in samples of malignant pleural effusion (the number of samples was not reported in the abstract). The malignancies tested were lung squamous cell carcinoma, adenocarcinoma, or small cell carcinoma, mesothelioma, breast cancer, lymphoma or leukaemia and 'miscellaneous'. Levels of CEA, AFP, CA153 and CA199 differed significantly between the different types of cancer, but CA125 did not. CA153 was the best biomarker for diagnosing malignant pleural effusions of lung adenocarcinoma (area under curve [AUC] 83.8%). At a cut-off of 10.2 U/ml, it had sensitivity of 73.2% and specificity of 85.2%. For lung squamous cell carcinoma, CA153 had an AUC of 71.6%; at a cut-off value of 14.2 U/ml it had sensitivity of 57.6% and specificity of 91.2%. For small-cell lung cancer, CA153 had an AUC 81.2%; at a cut-off value of 9.7 U/ml it had sensitivity of 61.5% and specificity of 94.1%. CEA was the best biomarker for diagnosing malignant pleural effusions of mesothelioma (AUC 72.6%); at a cut-off value of 1.43 ng/ml it had sensitivity of 83.7% specificity of 61.1%. For lymphoma or leukaemia CEA had AUC of 2.3%; at a cut-off value of 1.71 ng/ml it had sensitivity of 82.8% and specificity of 92.3%.

Topic expert feedback

No topic expert feedback was relevant to this evidence.

Impact statement

The evidence suggests that testing pleural effusion for cancer markers may not be a particularly efficient diagnostic strategy. The sensitivity for detecting various types of cancer was generally lower than immunohistochemistry performed directly on tumour samples. Therefore, an update in this area is not needed at this time.

New evidence is unlikely to change guideline recommendations.

RNA in-situ hybridisation

A study[22] assessed expression of albumin as a biomarker for intrahepatic cholangiocarcinoma in 467 samples of primary and metastatic cancers. RNA in-situ hybridisation was used to detect albumin expression. Overall, 83 samples were intrahepatic cholangiocarcinoma, 42 were hepatocellular carcinomas), and 332 were non-hepatic carcinomas including tumours arising from the perihilar region and bile duct, pancreas, stomach, oesophagus, colon, breast, ovary, endometrium, kidney, and urinary bladder. Albumin RNA in-situ hybridisation was positive in 99% of intrahepatic

cholangiocarcinomas and in 100% of hepatocellular carcinomas. Perihilar and distal bile duct carcinomas and carcinomas arising at other sites tested negative for albumin. Notably, 22% of 27 intrahepatic tumours previously diagnosed as CUP tested positive for albumin.

A study[23] assessed an RNA in-situ hybridisation assay compared with immunohistochemistry for detecting TTF-1 and napsin-A in 80 samples of lung adenocarcinoma and 80 samples of lung squamous cell carcinoma. The RNA in-situ hybridisation assay was also tested on 220 adenocarcinomas from various organs. The RNA assay for TTF-1 was positive in 92.5% of the lung adenocarcinomas, whereas immunohistochemistry was positive in 82.5% of lung adenocarcinomas. The RNA assay for napsin A gave positive results in 90% of lung adenocarcinomas, whereas immunohistochemistry was positive in 77.5%. The RNA assay for TTF-1 was positive in 10% of the lung squamous cell carcinomas, whereas immunohistochemistry was positive in 3.8% of lung squamous cell carcinomas. Napsin A expression was not seen in lung squamous cell carcinomas by either method. All RNA assay results in non-pulmonary adenocarcinoma were negative for TTF-1.

Topic expert feedback

No topic expert feedback was relevant to this evidence.

Impact statement

The evidence suggests that RNA in-situ hybridisation may be more sensitive than immunohistochemistry. However, only a few studies on a limited selection of biomarkers were identified, and there was no information about the specificity of this technique. Therefore, an update in this area is not needed at this time.

New evidence is unlikely to change guideline recommendations.

Q – 02 Gene profiling to identify the primary tumour in patients with provisional CUP or to guide treatment decisions in those with confirmed CUP

Recommendations derived from this review question

Gene-expression-based profiling

1.2.2.9 Do not use gene-expression-based profiling to identify primary tumours in patients with provisional CUP.

Surveillance decision

This review question should not be updated.

Molecular gene expression profiling

A study[24] included 141 people with CUP who had 'adequate tumour specimens' and received a single diagnosis after molecular profiling (CancerTYPE ID). The accuracy of diagnosis suggested by molecular profiling was assessed against later primary site discovery, initial immunohistochemistry, or further directed tests. Of 24 people who had later primary site discovery, molecular profiling correctly predicted the type of cancer in 75% of cases. Immunohistochemistry diagnoses matched molecular profiling diagnoses in 77% of cases. In cases that had further directed immunohistochemistry or clinical or histological testing, the molecular profile agreed in 74% of cases. Clinical features agreed with the molecular profiling diagnosis in 70% of cases.

A study[25] assessed a 92-gene reverse transcriptase polymerase chain reaction cancer classification assay in 289 people with untreated CUP. Patients then received treatment directed towards the predicted tissue of origin. Assays were successful in 87% of participants and 85% of participants received a predicted tissue of origin. The most commonly predicted sites of tumour origin were biliary tract (18%), urothelium (11%), colorectal (10%), and non-small-cell lung (7%). Site-specific treatment was used in 194 patients, who had a median survival time of 12.5 months. Median survival was significantly longer in tumours that were predicted to be clinically more responsive than in those whose tumours were predicted to be less responsive.

A study[26] assessed the effect of treatment of CUP after use of a 92-gene PCR-based assay (CancerTYPE ID). Of 1,544 patients who had assays performed, 125 people had predicted colorectal cancer. Surveys were sent to the treating physicians, and responses were received for 42 patients. Thirty-two patients received either first-line or second-line therapy with colorectal cancer chemotherapy regimens; the overall response rate was 50%. Patients who received first-line empirical therapy for CUP had an overall response rate of 17%. The median survival of patients who received site-specific therapy for colorectal cancer was 27 months compared with a historical survival of 8 to 11 months for people with CUP.

A prospective study[27] assessed the diagnostic accuracy of a 92-gene PCR-based assay compared with immunohistochemistry in 131 diagnostically challenging high-grade metastatic tumours. The final analysis included 122 cases. The 92-gene assay showed significantly greater tumour classification accuracy (79%) compared with immunohistochemistry and morphological analysis (69%) Immunohistochemistry used a mean of 7.9 stains per sample (range 2–15 stains).

A prospective blinded study[28] assessed gene expression profiling (Tissue of Origin) compared with immunohistochemistry in 157 samples of metastatic tumours from known primaries that represented the 15 tissues covered by the gene expression test. Immunohistochemistry testing was conducted by pathologists selecting from 84 stains in up to 2 rounds of testing. Overall, gene expression profiling accurately identified more specimens (89%), than immunohistochemistry (83%). In a subset of 33 poorly differentiated and undifferentiated carcinomas, gene expression profiling accurately identified significantly more specimens (91%) than immunohistochemistry (71%). In specimens for which pathologists rendered their final diagnosis with a single round of stains, both types of test exceeded 90% accuracy. However, when the diagnosis required a second round, immunohistochemistry was significantly less accurate than gene-expression profiling.

A study[26,29] compared immunohistochemistry compared with gene-expression profiling in 10 archived samples of cancer of known origin. Pathologists tested the samples with a selection from 84 immunohistochemistry stains with up to 2 rounds of testing; 5 pathologists each tested the 10 samples, which were added together to give 50 tests. The pathologist knew the patient's sex, biopsy site, and gross sample description, but were otherwise blind to the diagnosis. Gene-expression profiling (Tissue of Origin) was also performed. Gene expression profiling was accurate in 90% of tests and immunohistochemistry was accurate in 64% of tests.

A study[30] assessed a microarray based microRNA assay designed to identify 42 types of cancer in a validation set of 509 tumour samples. Overall assay sensitivity was 85%, and reached 90% for cases in which the assay reported a single answer. In 52 CUP samples, the assay showed 88% concordance with clinical and pathological findings.

A study[31] assessed a microarray using DNA methylation (EPICUP) for identifying the site of origin in CUP. First a 'training' sample of 2,790 samples of tumours of known origin were tested, representing 38 tumour types. The assay was then validated with 7,691 samples from the same types of tumour as the training sample. Finally, the assay was used to predict the site of origin in 216 samples of CUP. The accuracy of the assay was assessed using autopsy findings, later detection of a primary site, light microscopy and immunohistochemistry findings. The assay had sensitivity of 97.7%, specificity of 99.6%, positive predictive value of 88.6% and negative predictive value of 99.9% in the validation set of 7,691 samples. In the 216 CUP samples, the assay predicted a site of origin in 87% of 216 patients with cancer with unknown primary. Patients who received a tumour-type-specific

therapy showed improved overall survival compared with that in patients who received empiric therapy.

A prospective study[32] assessed 104 CUP samples using a microRNA assay that tests 48 targets. Overall, 87 of the samples contained enough tumour cells for testing and 74 samples were successfully tested. The assay-predicted site of origin was compared with clinical and pathological features and response to treatment. The assay result was consistent or compatible with the clinical and pathological features in 84% of the cases processed successfully. In 65 patients, pathology and immunohistochemistry suggested a diagnosis or a differential diagnosis. Out of those, the assay was consistent or compatible with the clinical and pathological presentation in 85% of cases. Of patients whose immunohistochemistry results were inconclusive, the assay provided site of origin prediction that was compatible with the clinical presentation most cases.

A study[33] assessed RNA sequencing with data from 17,471 transcripts from a total of 3,244 cancer samples across 26 different tissue types. An algorithm of cancer biomarkers was developed and then tested with a new set of randomly generated training and test sets. External validation of the cancer-specific signatures showed sensitivity of 92.0% and specificity of 97.7% for each cancer biomarker signature. Overall the algorithm showed accuracy of 90.5%.

A study[34] assessed a database integrating microarray- and sequencing-based gene expression profiles of 16,674 tumour samples covering 22 common types of tumour. From this database, a 154-gene expression signature was developed to determine the origin of tumour tissue with an overall accuracy of 96.5%. The 154-gene expression signature was validated on an independent test set consisting of 9,626 primary tumours, of which 97.1% of cases were correctly classified. An overall accuracy of 92% was achieved on 1,248 poorly differentiated, undifferentiated or metastatic tumour samples.

A study[35] assessed whether gene expression profiling could predict response to chemotherapy (everolimus plus carboplatin plus paclitaxel). A response rate of 22% was defined as successful treatment. The gene expression test was used to categorise tumours for which carboplatin plus paclitaxel is standard therapy and those for which this regimen is not standard therapy. Of 45 assessable patients, the response rate was 36%. Toxicities greater than grade 3 were predominantly haematological. Adequate tissue for gene expression profiling was available in 38 patients and predicted 10 different sites of origin. Patients with site of origin for which platinum plus taxane is standard therapy had significantly longer progression-free survival and overall survival compared with those whose site of origin would not usually be treated with platinum plus taxane chemotherapy.

A study[36] used a genomic profile assay (FoundationOne) to assess gene mutations in 125 CUP with features of adenocarcinoma and 75 CUP without features of adenocarcinoma. In 96% of samples, at least 1 mutation was found, with a mean of 4.2 mutations per tumour. In 85% of CUP specimens one or more of the gene mutations could have been amenable to targeted therapy. Mutations or amplifications of ERBB2 (HER2), EGFR, BRAF, and in the receptor tyrosine kinase and Ras signalling pathway were more frequent in CUP with features of adenocarcinoma than in CUP without features of adenocarcinoma.

Topic expert feedback

Topic expert feedback suggested that molecular gene expression profiling was increasingly clinically relevant.

Impact statement

Evidence on several commercially available gene profiling tests was identified, as well as other assays for which there was no information in the abstract to determine whether they are commercially available. Evidence generally shows that gene expression tests are associated with high rates of accuracy in classifying tumours.

A few studies directly compared gene expression testing with immunohistochemistry and appeared to be more accurate. However, these studies may not be a direct comparison of one technology over the other. In such tests, a set of gene expression tests was analysed by computer algorithm, for the comparison a human pathologist chose several immunohistochemistry tests from a large selection of possible targets. Such comparisons leave several questions unanswered:

- Is gene expression profiling using RNA inherently more accurate than antibody testing as used in immunohistochemistry?
- Is consistently testing a large panel inherently more accurate than selective testing of small panels?
- Do computer algorithms give more accurate predictions of tissue of origin than pathologists?

It is feasible that all these aspects influence the accuracy of the gene expression tests. The number of tests, their targets and the algorithms for predicting the site of origin will vary between the commercially available tests, but there were no comparisons to guide the choice of test.

Finally, one study bypassed the question 'What type of tumour is this?' to look directly for the presence of mutations known to influence response to treatments. However, there was no information on whether this affected patients' outcomes.

Although gene expression profiling shows promise, the evidence is insufficient to develop recommendations in this area at this time.

New evidence is unlikely to change guideline recommendations.

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