Overcoming the Unknown:
New Approaches to the Diagnosis and Treatment of Carcinomas of Unknown Primary.
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Diagnostic Strategies
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I would like to walk you down an overview of CUP diagnose. I am not going to go into the details of pathology and molecular profiling but will give an overview of how exactly we approach CUP patients. I will discuss the definition of CUP, talk a little about the primary and how far we need to go, pathological evaluation, where we stand with the immunohistochemistry and molecular diagnostics and then briefly discuss treatment.

As Dr Osborne very elegantly stated, we do not have a universal agreement on what the definition of CUP is. It is metastatic cancer in the absence of a radiographically or pathologically detectable primary tumour after an 'adequate' diagnostic evaluation. But we do not agree on what is 'adequate'. Historically it is 2 to 5% of all patients diagnosed with cancer. In the United States it is about 6th or 7th ranking, so it is among the first top ten cancers and the SEER database estimates it as 30,000 patients and that is an underestimate because it is surely grouped in a category of 'other unspecified primary sites' . It does not have a separate diagnostic classification in the SEER database and so it is underreported. The DRG coding is sometimes misreported because of the need for medications that need to be given to the patients and I would say that probably close to 70 – 80,000 patients in the US have CUP and to put that in perspective pancreatic cancer is 35, 000 and upper GI cancer is 25,000, so definitely there is a significant unmet need. At MB Anderson we see about 300 – 350 patients with CUP every year.

There is no sex predilection, the median age is 59 years, like most of the other solid tumour patients and the question is how far does one go in terms of looking for the primary. Again for a quick overview: everyone would agree that CT scans of the chest, abdomen and pelvis, when and where indicated, is something we all start with and other scans, including MRI of the brain or bone scan, should surely depend on symptomotology and patients pathology. Upper endoscopy, colonoscopy should be directed, based on patients pathology and presentation.

When do we do MRI's?
Clearly if someone has a CT iodine contrast allergy. It is clear that women who present with isolated axillary lymph nodes and have a negative mammogram and ultra sound are good candidates for a breast MRI. It is specific and it is helpful, because if it is negative it is going to show a very low yield at mastectomy, so it is best that these patients are then not taken in for surgery and are offered treatment for Stage 2 breast cancer with radiation to the breast when indicated.

When do we do PET scans?
Patients who come to see me come with more tests than I would have ordered and the question is: 'Do these patients really need PET scans?' When you start reviewing the PET scan literature what you find is that most studies are retrospective , they are small, and they are really not very clear in their indication as to whether the PET scan really helps us search for the primary and more so, does it impact treatment and survival? I think that there are two categories of patient where a PET scan is helpful:

1. A patient who has a squamous carcinoma and high cervical lymph node presentation, this group has been studied well and these patients are good candidates for PET because if you find a small primary in the head and neck area in a patient with squamous or cervical lymphadenopathy then it would help focus the radiation better and not just zap the whole neck and neck field, giving them chronic xerostomia. It also helps with future surveillance in these patients who do have a chance of second primaries. So head and neck cancers clearly.
2. Patients with single metastatic focus, who are surgical candidates. Somebody comes in with a small mass in the liver or one nodal site and surgery is a part of the consolidation plan, it would probably help getting a PET scan in such a patient because the extent of

disease is more than just that solitary focus. It clearly takes away a very aggressive curative plan, though one would definitely want to keep in mind that you want to put a lot of effort into biopsying the PET positive area, but it again depends on what the PET shows. So that group of patients with a solitary focus, where surgery is an option, is a good candidate for PET so that you can get a feel for the patients biology as well as start with a chemotherapy approach in such a patient.

3. Patients with disseminated disease: if you look at all the studies out there, the numbers you will see is 20 – 30% rate of detection of primary. Again, as I mentioned before, the studies are small and they are retrospective. The cost effectiveness of these studies is not clear and more often than not you find additional sites of metastases rather than the primary. At Anderson we do not have a PET IV contrast CT. We have the PET dye and we have the iodine but we have not been able to do both together. Our technicians tell us that there is a timing issue, so everybody gets an IV contrast CT scan and you make it a PET scan if you order that. At some point when we start doing these as one study it may be a very cost effective study.
   This slide shows data on a survey on PET in Cervical CUP presenting with squamous cell neclanflanophany and you can see that the studies are small but there is a suggestion that it could help these patients.

Pathological evaluation:
A patient who has CUP comes in with a biopsy of a metastatic focus, the cancer goes through various pathologic evaluations, and Dr Oien will discuss this in detail, but for an overview: microscopy and immunohistochemistry are routinely used. We rarely do chromosome studies or electron microscopy and now we have the emerging techniques of molecular diagnostics available.

An H&E of CUP cancer is usually a NADNo carcinoma, a squamous cancer, poorly differentiated cancer, neuroendocrine or just an undifferentiated neoplasm. Patients who have sarcoma, lymphoma, melanoma presenting as a CUP in a nodal disease - because there is a histological and stage specific treatment for these patients we do not keep them in the CUP clinic. These patients are best served by a melanoma specialist, or a sarcoma specialist. So most of the patients we see have adenocarcinoma or squamous cell or mixed tumours.

- 60% of patients will have adenocarcinoma like microscopy
- 5% pure squamous cell, which is not all that common
- 30% is a mixed bag of poorly differentiated 'neoplasm', poorly differentiated adenocarcinoma or just a very poorly differentiated carcinoma.

Following H&E the tumour goes through immunohistochemical stains. These stains are important in defining tumour lineage. They are often immunoperoxidase stains and it is a peroxidise labelled antibody against a specific tumour antigen. Then it goes through various tiers of immunohistochemistry. This is actually the best way of doing it but it's really not very uniform between institutions in the United States and in the community in the United States; some people get just two and some may get forty stains on these patients so it is not very clear how exactly one should tier the immunohistochemical stains so that it is cost effective but gives you as much information as you can get in the tier system.

There are potential problems with stains: you need technical expertise, you need very experienced pathologists to interpret the results, one should avoid over interpretation - there is no staining pattern that is entirely specific, even PSA can be positive in a salviary cystic carcinoma - and most importantly communication between a clinician and pathologist is essential. Every time I have called up a pathologist and discussed a CUP case I have always learnt something new and helped the pathologist narrow it down a little bit, because I have all the clinical information on the patient and the pathologist just has a tiny piece of tissue.

We often start with our first tier stains:

- Most of our patients with adenocarcinoma get a CK7, CK20 after we have established this is an epithelial carcinoma.

- A patient who has CK20, CDX2 and a CK7 negative profile has a cancer that behaves like a colorectal cancer and we will go into that in detail as we go through the meeting.
- Patients with CK7+ and CK20- show a huge differential. We really don't know sometimes. The second tier stains can help us, as can a patients risk factors, gender, pattern of presentation but it is still a very large differential.
- Strongly positive CK7 and CK20 pathology point to urothelial tumours, ovarian cancers, pancreatic carcinoma, cholangiocarcinoma profiles. I like to call them profiles because clearly there is no primary cancer, otherwise the patient wouldn't be in my CUP clinic.
- Negative CK7 and CK20 point to hepatocelluar carcinoma and renal cell carcinoma.

Immunohistochemical stains give us clues; they are not Gold Standard in making a diagnosis.

The second tier stains used are:

- TTF-1 is something we look at for lung cancer. 60 – 75% of adenocarcinomas are TTF-1 positive.
- GCDFP-15 , which is Gross Cystic Disease Fluid Protein, 60% of the time in breast cancer. Again if it is very poorly differentiated cancer these stains can be negative.
- Calretinin is used for mesothelioma
- UROIII is a transitional cell marker
- CDX02 is a good GI Tract Cancer marker
- Chromogranin and synaptophysisn for Neuroendocrine cancers.

Every time I mention Electron Microscopy my pathologists are upset because they do not like doing electron microscopy and tell me that it is dated and we really do not have electron microscopist specialists in the United States any more. It is a very time consuming process and takes a good ten to twelve days, at least, to get the report back and you often have to make a decision sooner. It shows you the ultra structural details of the cell, so in a squamous cell you would see desmosomes and tonofilaments, microvilli for adenocarimona, core granules for neuroendocrine and the pictures are very pretty. But again the question is: in which patient will this be helpful? And that is not clearly defined.

There are also various emerging techniques of molecular diagnostics: Let me first take a step back and talk about the biology and where are we going with this right now.

Does it really matter to identify the tissue of origin? There are two prevailing hypotheses about CUP:

- Is it a single biological entity? Is it something with a unique molecular and biochemical basis? Or
- Is it a heterogeneous group of unrelated cancers, which are site specific tumours that just happen to share the property of having a diminutive primary that escapes detection?

It is very possible that the truth is somewhere in between but we do not have those unique traits that we can use as targets for treatment at this time and the scale has tipped towards the second hypothesis. Having profiling and finding out what site specific tumour it may be may be helpful.

With newer and more sensitive imaging tools primaries are being identified in patients initially diagnosed with CUP. One gentleman mentioned here that we are finding latent primaries. Perhaps the next test was a more sophisticated test that showed a latent primary or a very tiny tumour that was growing over time. With longer survival we are finding some latent primaries, so clearly these patients do have small primaries, at least a percentage of them, that then may show up over time, although that is not very common.

With molecular profiling and Immunohistochemical studies we may identify distinct subsets, whose profiles may match known metastatic lesions and this really matters when you have chemotherapy that works. So chemotherapy regimens active against anatomically defined cancers, like colon cancer, can be applied to appropriate CUP subgroups.

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If the case is that it matters where the cancer came from, then the current front line treatments using doublet cytotoxic therapies may be a 'one fit all' management and we may need to take a more targeted and individualised approach to CUP and that may hold more promise; for example a colon profile of unknown primary, or patients with isolated carcinomatosis who may have a very specific subtype. The exception may be the favourable subtypes.

This slide is an example of patients with peritoneal carcinomatosis presenting with CUP. You can have very bulky disease, this patient did not have PMP and you can have a patient with more stranding and nodularity . If you present with CUP with carcinomatosis clearly upper endoscopy and colonoscopy in these patients was negative. It is a huge differential. It could be in a woman, a primary peritoneal carcinoma, ovarian, Signet ring cell, non signet stomach cancer, colon, pancreas, cholangio, appendiceal , sometimes a mesothelioma that may be mislabelled as carcinoma. So using a very robust immunochemical platform and emerging profiling probes we hope to individualise therapy for this entity.

The treatments matter. If somebody had isolated carcinomatosis and has a pancreatico billiary profile to the carcinomatosis it may be a germcitary based regimen. If it is a gastric profile it could be a gastric and oesophageal type regimen. Colorectal appendacial profile gets ???????????? types and biologics. So the treatments matter, based on what the subtype is.

The idea is to move away from Phase II studies evaluating 'empiric' combinations and focus understanding on the CUP biology and predicting the tissue of origin. Using this information we can then leverage promising treatments for known cancers. Clearly the approach is limited at this time because of the paucity of effective drugs for several cancers. It does not really matter whether it is pancreas or billiary, a patient can get ????????????? based therapy but if you do have a treatment that works it does matter. For kidney cancer it matters right now to know whether it is renal cell because I am not going to give the patients hydrotoxics, the patient is going to get a targeted therapy ceraphenibs ,so it will matter as we go along and as normal targeted and cytotoxic therapies are developed for site specific cancers they should be evaluated in an appropriate CUP subtype.

Briefly there are limitations to the pathologic diagnostic modalities in CUP:

- Immunohistochemistry and profiling limitations
- During the day we will discuss the variabilities involved
- Expense needs to be considered
- The heterogeneity of the histologic subtypes in profiling
- Most importantly do these tests impact survival?

The current CUP management approach is looking at imaging studies, immunohistochemistry and patients patterns of presentation. I am hopeful that molecular profiling, over the next few years, becomes a standard, integral part, of working up CUP patients and we put this puzzle together and even more so, my dream is that over time we can start coming up with subtypes within molecular profiles. Patients who present with isolated osseous metastases – why do they get only bone tumours? Bone profile, carcinomatosis profile, colon profile and we can then dissect out these subtypes and treat patients with very effective, site specific treatments that can then help our patients with CUP subtypes.

Thank you very much for your attention.